IN THE UNITED STATES DISTRICT COURT FOR THE DISTRICT OF MASSACHUSETTS

PETER J. MILLER, an individual, CLIFFORD HOYT, an individual, and CAMBRIDGE RESEARCH AND INSTRUMENTATION, INC., a Massachusetts corporation,	
Plaintiffs,	
v.	Civil Action No. _____
PATRICK TREADO, an individual, and CHEMIMAGE CORP., a Delaware corporation,	
Defendants.	

MAGISTRATE JUDGE_____

RECEIPT # _____
AMOUNT $_____
SUMMONS ISSUED _____
LOCAL RULE 4.1 _____
WAIVER FORM _____
MCF ISSUED _____
BY DPTY. CLK _____
DATE _____

COMPLAINT

Plaintiffs Cambridge Research and Instrumentation, Inc. ("CRI"), Peter J. Miller, and Clifford Hoyt hereby complain against defendants ChemImage Corporation ("ChemImage"), assignee of U.S. Pat. No. 6,734,962 ("the '962 patent"), and Patrick Treado, named co-inventor of the '962 patent, for actions as described below and request relief from the court, specifically, that the court (1) declare plaintiffs Peter J. Miller and Clifford Hoyt co-inventors of the '962 patent, and order appropriate correction thereof; (2) declare the '962 patent unenforceable because of inequitable conduct; (3) declare any patent(s) issuing from either U.S. Patent Application Serial No. 10/773,077 or U.S. Patent Application Serial No. 10/610,481 unenforceable because of inequitable conduct; (4) declare one or more claims of the '962 patent invalid as anticipated, and/or rendered obvious, by sales and/or offers for sale by defendant ChemImage; and (5) provide further relief as indicated below; and (6) provide such other relief as the court deems just and necessary.

PARTIES

1.   Plaintiff Cambridge Research and Instrumentation, Inc. ("CRI") is a corporation incorporated under the laws of the State of Delaware and has its principal place of business in the State of Massachusetts. Founded in 1985, CRI develops and sells precision equipment for the measurement and control of light. Such equipment includes, but is not limited to, liquid crystal tunable filters, microscopes, and spectrometers.

2.   Plaintiffs Peter J. Miller and Clifford Hoyt are individuals residing in the State of Massachusetts, and are presently employed by CRI, and have been employed at all times relevant herein by CRI.

3.   Upon information and belief, defendant ChemImage Corporation ("ChemImage") is a corporation incorporated under the laws of the State of Delaware and has its principal place of business in Pittsburgh, Pennsylvania. ChemImage sells, among other things, Raman imaging microscopy systems.

4.   Upon information and belief, defendant Patrick Treado is an individual residing in the State of Pennsylvania, is presently president of ChemImage, Inc., and has been president of ChemImage since at least 1994.

5.   Upon information and belief, defendant ChemImage was previously called ChemIcon, Inc. If the corporation known as ChemImage is not the present business incarnation of ChemIcon, Inc., it is believed that ChemImage is, for all intents and purposes herein, the same business entity as ChemIcon, Inc. as it has the same president, defendant Patrick Treado, and has continued in the same business as ChemIcon, Inc.

JURISDICTION

6.   This is an action for a declaratory judgment pursuant to the Federal Declaratory Judgments Act, 28 U.S.C. §§2201 and 2202. This action arises, at least in part, under the patent laws of the United States, 35 U.S.C. §1 et seq., and the court has jurisdiction over this action pursuant to 28 U.S.C. §1338(a).

BACKGROUND

7.   In June 1994, plaintiff CRI filed a Small Business Innovation Research (SBIR) Phase I Proposal entitled "High Definition Raman Imaging Microscope" with the National Science Foundation (NSF) (Exhibit A). Plaintiff Peter J. Miller was listed as the Principal Investigator/Project Director (PI) in the SBIR Phase I Proposal; plaintiff Clifford Hoyt was identified as a "[k]ey CRI researcher" in the project. Defendant Patrick Treado was listed as a consultant in the SBIR Phase I Proposal. Defendant Patrick Treado assisted in the preparation of the SBIR Phase I Proposal.

8.   The Phase I Proposal proposed building a chemical imaging system having (a) an illumination source; (b) infinity-corrected optics for collecting and collimating light from an area illuminated by the light source; (c) a Lyot-based liquid crystal tunable filter (LCTF) for an imaging spectrometer; and (d) a CCD camera for collecting images from the imaging spectrometer.

9.   The Phase I Proposal states that "[t]he first use of LCTF for Raman Microscope (*sic*) was performed at the University of Pittsburgh in a collaborative effort between CRI researchers and Prof. Treado." (See Sect. E.3, Exhibit A).

10.   At least as early as 1993, plaintiff Clifford Hoyt conceived the idea of using "infinity space," or infinity-corrected optics, in a Raman imaging microscope using an LCTF, and at least as early as 1993 conveyed that idea to defendant Patrick Treado.

11.   The Phase I Proposal contemplated using a Lyot-based filter design. However, the Phase I investigation found that a Lyot-based design did not perform well, and a new design based on an Evans split-element retarder filter was conceived and reduced to practice by plaintiff Peter J. Miller. As stated in the Final Report for Phase I (Exhibit B), "[t]he principal limitation of [the Lyot-based] design is its relatively low transmission, which ranges from 7 to 16 percent. A novel design was devised to overcome this problem, and a model was developed of this new design. Based on the Evans split-element retarder, it should nearly treble the throughput of the LCTF, as described in Section IV, Key Improvements for Phase II." (Exhibit B, page 5).

12.   In October 1996, plaintiff CRI filed a SBIR Phase II Proposal entitled "High

Definition Raman Imaging Microscope" with the NSF (Exhibit C). The SBIR Phase II Proposal again listed plaintiff Peter J. Miller as the Principal Investigator/Project Director (PI), and defendant Patrick Treado as a consultant. Defendant Patrick Treado assisted in the preparation of the SBIR Phase II Proposal.

13. As stated therein, the proposed Phase II research would "build on the successful imaging Raman instrument demonstrated in Phase I" and, specifically, that "key improvements will be made: transmission will be doubled (or more) based on a high-efficiency design identified in Phase I, and the long-wavelength limit will be extended from the present 700 nm to 1050 nm. Such an LCTF appears to offer near-revolutionary benefits in certain applications including semiconductor analysis, biomedical imaging, and pharmaceutical research." (Exhibit C, page C-1).

14. Section M.4 of the Phase II Proposal, labeled "Justification of Consultant", states that "Dr. Treado will be principally responsible for the task of meeting objective iv), the evaluation of applications targeted as having commercial potential" and that this task will involve "imaging Raman analysis of various samples relevant to the target applications." (Exhibit C, page 25). A curriculum vitae for defendant Patrick Treado provided in the SBIR Phase II Proposal states that "[i]n 1994, Prof. Treado founded Chemicon, Inc., a company dedicated to commercializing chemical imaging technologies, including Raman microprobes for continuous process monitoring, fluorescence imaging systems to support drug discovery and Raman imaging microscopes." (Exhibit C, page 17).

15. The SBIR Phase II Proposal further states that "ChemIcon has already introduced a line of Raman microscopes based on the Phase I prototype, and have (*sic*) quotations pending with several customers totaling over $300K" (Exhibit C, Appendix 2A).

16. A letter dated October 24, 1996 written "[i]n support of CRI's application for NSF Phase II SBIR funding" was signed by both defendant Patrick Treado, as president of ChemIcon, and Peter V. Foukal, president of plaintiff CRI (Exhibit D). The letter was a "Follow-On Funding Commitment letter" in which ChemIcon agreed to commit funding for the SBIR Phase II proposal in return for a "time-limited exclusive license" of 12 months for any innovative technology resulting therefrom, after which CRI would have the right to sell the new technology to other customers. The letter states that it is "anticipated, at this time,

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that innovations that will result from the Phase II research will include, but will not be limited to, the development of red-wavelength optimized Raman LCTFs, and high throughput (Split Evans) design Raman LCTFs."

17.   A letter written by defendant Patrick Treado and dated October 28, 1996 was appended to the SBIR Phase II Proposal (Exhibit E). In this letter, defendant Patrick Treado states that "CRI's liquid crystal tunable filter (LCTF) technology represents a revolutionary advancement in imaging spectrometer technology that is ideally suited to Raman imaging microscopy" and that his role in the Phase II project was "to assist in the development of liquid crystal tunable filters (LCTFs) and their application to Raman imaging".

18.   Prior to October 13, 1999, plaintiff CRI built and sold to defendant ChemImage or its predecessor Evans split-element filters optimized for use in Raman imaging microscopes using near infrared radiation. Upon information and belief, on or before October 13, 1999, defendant ChemImage or its predecessor offered for sale and/or sold Raman imaging microscopes incorporating said Evans split-element liquid crystal tunable filter.

19.   On October 13, 2000, provisional patent application serial number 60/239,969 ("the provisional application") entitled "Near Infrared Chemical Imaging Microscope" was filed with the United States Patent and Trademark Office (PTO) listing defendant Patrick Treado, Matthew Nelson, Scott Keltzer, and Juliana Riber as co-inventors.

20.   The provisional application contained over 300 pages of material, including many lab notebooks. None of the lab notebook pages appears to be dated before 1999.

21.   On October 12, 2001, a regular patent application serial number 09/976,391 ("the '391 application") entitled "Near Infrared Chenical Imaging Microscope" and claiming priority from the provisional application was filed with the PTO, listing defendant Patrick Treado, Matthew Nelson, and Scott Keltzer as co-inventors.

22.   Independent claim 1 of the '391 application as filed recites a chemical imaging system using near infrared radiation comprising (a) a near infrared illumination source, (b) a device for collecting and collimating light from an area illuminated by the illumination source, (c) a near infrared imaging spectrometer for selecting images of said collimated

light, and (d) a detector for collecting the images.

23. Claim 3 of the '391 application recites that the collecting/collimating device (b) in claim 1 may comprise, among other things, a "refractive type infinity-corrected near infrared optimized microscope objective."

24. Claim 4 of the '391 application recites that the imaging spectrometer (d) in claim 1 may comprise, among other things, an "Evans Split-Element liquid crystal tunable" filter.

25. In an assignment dated January 2004 and recorded at Reel/Frame 014302/0906 at the PTO, Matthew Nelson, Scott Keltzer, and defendant Patrick Treado assigned their rights in the '391 application to defendant ChemImage.

26. At no point during the prosecution of the '391 application did defendants ChemImage and Patrick Treado inform the PTO of the previous collaborative work with CRI on Raman imaging microscopes with integrated Evans split-element filters and collected collimated light, or the offers for sale made on the microscope based on the Phase I prototype. Nor did defendants ChemImage and Patrick Treado inform plaintiffs CRI, Peter J. Miller, or Clifford Hoyt of either the provisional application or the '391 application.

27. On May 11, 2004, the '391 application issued as U.S. Patent Number 6,734,962 ("the '962 patent"). (Exhibit F)

28. Before the '391 application issued as the '962 patent, defendants ChemImage and Patrick Treado filed continuation patent application serial number 10/773,077 ("the '077 application") and continuation-in-part patent application serial number 10/610,481 ("the '481 application"), both of which claim priority from the provisional application and the '391 application.

29. Promptly upon learning of the '962 patent, plaintiff CRI contacted defendant ChemImage to express CRI's concerns regarding the issue of inventorship in an effort to negotiate a settlement of that issue that would avoid litigation. On December 29, 2004, CRI sent a letter to ChemImage presenting CRI's views on the inventorship issue (Exhibit G). In response, counsel for defendant ChemImage mailed a letter dated January 20, 2005 (Exhibit H), in which it was asserted that:

> While the SBIR proposal suggests that Peter Miller and Dr. Treado

collaborated on certain technology, much of the subject matter described
in the SBIR proposal was invented by Dr. Treado before any such
collaboration took place. The fact that Peter Miller and Dr. Treado may
have collaborated on ideas previously invented by Dr. Treado does not in
any way diminish Dr. Treado's position as the sole inventor of the
technology that he (and ChemIcon) brought to the SBIR proposal"
(Exhibit H, page 1).

COUNT 1: CORRECTION OF INVENTORSHIP

30. Plaintiffs repeat and reallege the assertions set forth in paragraphs 1 through 29.

31. Plaintiff Peter J. Miller, either individually or in collaboration with defendant
Patrick Treado, contributed to the invention of the subject matter of one or more claims in
the '962 patent, including, for example, Miller's conception of the use of an Evans Split-
Element liquid crystal tunable filter in a chemical imaging system using near infrared
radiation as recited in claim 4 of the '962 patent.

32. Plaintiff Clifford Hoyt, either individually or in collaboration with defendant
Patrick Treado, contributed to the invention of the subject matter of one or more claims in
the '962 patent, including, for example, Hoyt's conception of the use of a refractive type
infinity-corrected microscope objective as the collecting/collimating device in a chemical
imaging system using near infrared radiation as recited in claim 3 of the '962 patent.

33. Wherefore plaintiffs ask the court (a) to declare that Peter J. Miller is a co-
inventor of the subject matter of one or more of the claims in the '962 patent and (b) to
order correction of the '962 patent under 35 U.S.C. §256 to reflect Peter J. Miller as a co-
inventor.

34. Wherefore plaintiffs ask the court (a) to declare that Clifford Hoyt is a co-
inventor of the subject matter of one or more of the claims in the '962 patent and (b) to
order correction of the '962 patent under 35 U.S.C. §256 to reflect Clifford Hoyt as a co-
inventor.

COUNT 2: UNENFORCEABILITY OF THE '962 PATENT
FOR INEQUITABLE CONDUCT IN MISREPRESENTING INVENTORSHIP

35.   Plaintiffs repeat and reallege the assertions set forth in paragraphs 1 through 34.

36.   One or more persons with a duty of candor to the PTO during the prosecution of the '962 patent, such as defendant and named inventor Patrick Treado, knew that the subject matter of one or more claims in the '962 patent was co-invented by Peter J. Miller, at least as a consequence of defendants ChemImage and Patrick Treado's collaboration with plaintiff Peter J. Miller on the work described in the SBIR Phase I and Phase II Proposals.

37.   Such one or more persons, such as defendant and named inventor Patrick Treado, failed to inform the PTO of Peter J. Miller's co-inventorship, and, upon information and belief, such failure was in bad faith and/or with deceptive intent.

38.   One or more persons with a duty of candor to the PTO during the prosecution of the '962 patent, such as defendant and named inventor Patrick Treado, knew that the subject matter of one or more claims in the '962 patent was co-invented by Clifford Hoyt, at least as a consequence of defendants ChemImage and Patrick Treado's collaboration with plaintiff Clifford Hoyt on the work described in the SBIR Phase I and Phase II Proposals

39.   Such one or more persons, such as defendant and named inventor Patrick Treado, failed to inform the PTO of Clifford Hoyt's co-inventorship, and, upon information and belief, such failure was in bad faith and/or with deceptive intent.

40.   Wherefore plaintiffs ask the Court to declare the '962 patent unenforceable for inequitable conduct.

COUNT 3: UNENFORCEABILITY OF THE '962 PATENT
FOR INEQUITABLE CONDUCT IN FAILING TO DISCLOSE MATERIAL INFORMATION

41.   Plaintiffs repeat and reallege the assertions set forth in paragraphs 1 through 40.

42.   The contributions of plaintiffs Peter J. Miller and Clifford Hoyt to the collaboration with defendants ChemImage and Patrick Treado on the work described in the SBIR Phase I and Phase II Proposals ("the CRI/ChemIcon collaboration"), either by itself or in combination with other information, establishes a *prima facie* case of unpatentability

of one or more claims in the '962 patent.

43.   One or more persons with a duty of candor to the PTO during the prosecution of the '962 patent, including at least defendant and named inventor Patrick Treado, knew of the CRI/ChemIcon collaboration.

44.   Such one or more persons, including at least defendant Patrick Treado, failed to inform the PTO of the CRI/ChemIcon collaboration and, upon information and belief, such failure was in bad faith and/or with deceptive intent.

45.   The offer of sale and/or sale by ChemIcon of Raman microscopes based on the Phase I prototype, either by itself or in combination with other information, establishes a *prima facie* case of unpatentability of one or more claims in the '962 patent.

46.   One or more persons with a duty of candor to the PTO during the prosecution of the '962 patent, including at least defendant Patrick Treado, knew of ChemIcon's offer for sale and/or sale of the Raman microscopes based on the Phase I prototype.

47.   Such one or more persons failed to inform the PTO of such offer for sale and/or sale and, upon information and belief, such failure was in bad faith and/or with deceptive intent.

48.   The offer of sale and/or sale by defendant ChemImage or its predecessor of Raman imaging microscopes incorporating Evans split-element filters made by plaintiff CRI, either by itself or in combination with other information, establishes a *prima facie* case of unpatentability of one or more claims in the '962 patent.

49.   One or more persons with a duty of candor to the PTO during the prosecution of the '962 patent, including at least defendant Patrick Treado, knew of ChemImage's or its predecessor's offer for sale and/or sale of Raman imaging microscopes built with plaintiff CRI's Evans split-element filters.

50.   Such one or more persons failed to inform the PTO of such offer for sale and/or sale and, upon information and belief, such failure was in bad faith and/or with deceptive intent.

51.   Wherefore plaintiffs ask the Court to declare the '962 patent unenforceable for inequitable conduct.

COUNT 4: UNENFORCEABILITY OF THE '077 AND '481 APPLICATIONS
FOR INEQUITABLE CONDUCT IN THE PARENT '092 PATENT

52.   Plaintiffs repeat and allege the assertions set forth in paragraphs 1 through 51.

53.   The intentional misrepresentation of inventorship of the subject matter claimed in the '962 patent taints any application or patent related to the '962 patent which claims, or at any point during prosecution claimed, subject matter related to the subject matter co-invented by the unnamed co-inventors Peter J. Miller and Clifford Hoyt.

54.   The intentional failure to disclose material information during prosecution of the '962 patent taints any application or patent related to the '962 patent which claims, or at any point during prosecution claimed, subject matter related to such undisclosed material information.

55.   Both the '077 and the '481 applications claim subject matter related to the subject matter in the '962 patent which was co-invented by the unnamed co-inventors Peter J. Miller and/or Clifford Hoyt, and/or related to subject matter in the '962 patent related to the material information which defendants withheld from the PTO.

56.   Wherefore plaintiffs ask the court to declare any patent related to the '962 patent which claims, or at any point during prosecution claimed, tainted subject matter, including any patent(s) issuing from the '077 application and the '481 application, to be unenforceable as a consequence of the inequitable conduct which occurred during prosecution of the '962 patent.

COUNT 5: INVALIDITY OF THE '962 PATENT
UNDER THE STATUTORY BAR OF 35 U.S.C. §102(b)

57.   Plaintiffs repeat and reallege the assertions set forth in paragraphs 1 through 56.

58.   The offer of sale and/or sale by ChemIcon of Raman microscopes based on the Phase I prototype, which, upon information and belief, occurred before October 13, 1999, renders one or more claims, including, but not limited to, claims 1, 3, and 4, of the '962 patent, invalid under 35 U.S.C. §102(b) and/or 35 U.S.C. §103.

59.   The offer for sale and/or sale by defendant ChemImage or its predecessor of

Raman imaging microscopes incorporating the Evans split-element filters made by plaintiff CRI, which offer for sale and/or sale, upon information and belief, occurred before October 13, 1999, renders one or more claims, including, but not limited to, claims 1, 3, and 4, of the '962 patent, invalid under 35 U.S.C. §102(b) and/or 35 U.S.C. §103.

60.   Wherefore plaintiffs ask the Court to declare one or more claims, including, but not limited to, claims 1, 3, and 4, of the '962 patent, invalid as anticipated under 35 U.S.C. §102(b) and/or as obvious under 35 U.S.C. §103.

WHEREFORE, plaintiffs respectfully request that this Court:

- on Count 1, declare plaintiffs Peter J. Miller and Clifford Hoyt co-inventors of the '962 patent and order correction of the '962 patent under 35 U.S.C. § 256 to reflect their status as co-inventors;

- on Counts 2 or 3, declare the '962 patent unenforceable;

- on Count 4, declare any patent(s) issuing from the '077 and '481 applications unenforceable;

- on Count 5, declare at least claims 1, 3, and 4 of the '962 patent invalid for anticipation under 35 U.S.C. §102(b) and/or obviousness under 35 U.SC. §103;

- declare this an exceptional case pursuant to 35 U.S.C. § 285 and award plaintiffs their reasonable attorneys fees;

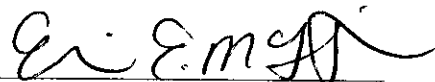- award plaintiffs their costs; and

- such other and further relief as this Court shall deem just and proper.

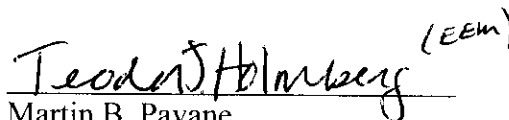   Respectfully submitted,

                                    PETER J. MILLER, CLIFFORD HOYT, and CAMBRIDGE RESEARCH AND INSTRUMENTATION, INC.,

                                    By their attorneys:

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Dated: February 24, 2005

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Return to Hoyt

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National Science Foundation
Small Business Innovation Research Program

PROJECT SUMMARY

	NSF AWARD NO.

NAME OF FIRM
Cambridge Research & Instrumentation, Inc.

ADDRESS
21 Erie Street
Cambridge, MA 02139

PRINICPAL INVESTIGATOR (NAME AND TITLE)
Peter Miller, Staff Scientist

TITLE OF PROJECT
High Definition Raman Imaging Microscope.

TOPIC TITLE	**TOPIC NUMBER AND SUBTOPIC LETTER**
Chemistry	2.b.

PROJECT SUMMARY

Development of the high definition liquid crystal tunable filter (LCTF) described in this proposal will unleash the potential of Raman chemical imaging microscopy for non-invasive chemical characterization of solid-state materials. The LCTF will allow Raman chemical imaging to become a mainstream analytical methodology for the first time, accessible even to non-experts and applicable for routine industrial process monitoring and materials analysis.

Raman chemical imaging has the capability to characterize heterogeneous systems without the need for significant sample preparation. Chemical imaging allows one to visualize the composition and spatial distribution of constituents that dictate material function, which is fundamental to characterizing advanced composite materials. An optimized Raman imaging filter based on liquid crystals can readily be used to analyze a wide variety of materials, including polymers, corrosion resistant alloys, and pharmaceuticals.

In Phase I, we will demonstrate feasibility by constructing a high resolution LCTF and using it in a microscope to obtain Raman images from test samples. Phase II will involve LCTF optimization and integration into a turnkey high definition Raman microscope consisting of a laser, microscope, LCTF, and CCD detector, together with the appropriate analysis and processing software. C.R.I. has a proven track record commercializing SBIR technology in Phase III.

Potential Commercial Applications of the Research

A Raman chemical imaging system will have broad applicability in the polymer and coatings industries for chemically specific visualization of domains and defects without the need for sample staining. In-situ monitoring of corrosion in ferrous alloys is another important use, while a third application lies in quantitative studies of polymorphism in pharmaceutical crystalline materials.

KEY WORDS TO IDENTIFY RESEARCH OR TECHNOLOGY (8 MAXIMUM)

Raman, chemistry, spectroscopy, imaging, microscopy

D.   Identification and Significance of the Problem or Opportunity
Development of the high definition liquid crystal tunable filter (LCTF)
described in this proposal will unleash the potential of Raman chemical
imaging microscopy for non-invasive chemical characterization of solid-state
materials. The performance advantages provided by LCTFs will allow Raman
chemical imaging to become a mainstream analytical methodology for the first
time, accessible even to non-experts and applicable for routine industrial
process monitoring and materials analysis.

Raman chemical imaging is an emerging but vital area in chemistry that
integrates Raman spectroscopy with imaging technology. Chemical imaging
provides the ability to visualize the chemical composition, concentration and
dynamics of specific constituents within composite materials, living cells and
tissues. Chemical imaging is an interdisciplinary field in its methodology and
so it can impact many important areas of science and medicine. Examples of
chemical imaging include nuclear magnetic resonance imaging (MRI) employed for
clinical diagnosis, and satellite remote sensing for global imaging of ozone
depletion.

High-resolution Raman chemical imaging has the potential for characterizing
heterogeneous systems rapidly and non-invasively without the need for
significant sample preparation. Chemical imaging can visualize *in situ* the
composition and spatial distribution of constituents that dictate material
function. Understanding the structure/function relationship is fundamental to
the characterization of advanced composite materials. The development of an
optimized Raman imaging filter based on liquid crystals can readily be applied
to the characterization of a wide variety of materials, including polymers,
corrosion resistant alloys, and pharmaceuticals.

Raman spectroscopy on the microscopic scale has shown tremendous power as a
tool for chemical analysis since the development of the first practical Raman
microprobe, a technique which employs a diffraction grating coupled to a CCD
detector for rapid collection of Raman microspectra from a single, tiny spot
on the sample [1]. Raman spectroscopy is attractive because it provides an
almost universally applicable chemically-selective means of contrast
generation by relying on the vibrational spectrum intrinsic to a material,
without the need for stains or dyes. In addition, high spatial resolution (~
1 μm) is readily achievable when this technique is combined with optical
microscopy.

However, Raman imaging presents significant experimental challenges. The
Raman scattering process is inefficient; on average only 1 in 10^8 incident
photons scatter inelastically, so Raman microscopy produces signals with low
light-levels. This places stringent requirements on the components employed.
A number of technological innovations in recent years make it feasible to
perform non-imaging Raman spectroscopy routinely. These innovations include
high dynamic range charge-coupled device (CCD) detectors and holographic
optics for laser light rejection. Despite these innovations, Raman imaging
remains cumbersome because of the lack of a high spectral resolution, broad
free spectral range, electronically tunable spectrometer technology that
simultaneously transmits entire Raman images with high clarity.

3

The power of Raman spectroscopy is well established for non-imaging characterization of materials, including polymers, semiconductors, biological tissues, catalytic surfaces, corrosion-resistant alloys [2-4]. In polymer studies, for example, Raman spectroscopy is useful for the analysis of chemical composition and structure. The technique can differentiate between internal and external bonds, cis- and trans-isomerism and conjugation. In addition, the helical conformation of polymer chains in the solid-state can be monitored. Raman imaging microscopy provides the ability to visualize polymer chemistry. Internal defects and voids that appear invisible using conventional light microscopy techniques can be characterized with molecular specificity using Raman imaging. Molecular interactions between heterogeneous materials and the homogeneity of mixing processes can also be observed.

The development of broadly tunable, high resolution liquid crystal tunable filter (LCTF) imaging spectrometers as described here will allow Raman microscopy to evolve beyond the scientific curiosity stage, practiced mostly in academic research laboratories, into a powerful technique for materials characterization and industrial process monitoring.

E.   Background and Technical Approach

E.1   Background and Technical Approach
Raman imaging provides the ability to visualize samples non-invasively and the methodology requires a tunable filter technology that provides high spatial and spectral resolution. LCTF technology is the only approach that simultaneously provides a broad spectral range, fine spectral resolution, wide acceptance angle, and large optical aperture, while providing high out-of-band rejection. This technology is physically compact, mechanically rugged and electronically controllable without the need for moving mechanical parts.

LCTFs are versatile devices which can function from the visible to the near-infrared, with prospects for operation in the mid-infrared. C.R.I. has pioneered the development of LCTFs, based on nematic liquid crystal technology [5]. These instruments incorporate liquid crystal optical retarders within Lyot birefringent filters, to provide an electronically tunable spectral passband, as shown in Figure 1. LCTFs have large acceptance angles and can be fabricated with large optical apertures. They provide acceptable peak transmittance (15-20%), narrow bandpass (to 0.2 nm), and rapid switching speeds (50 msec). Their out-of-band rejection (10^4) is the highest of any viable tunable filter technology. The throughput, versatility, and spectral purity of the LCTF make it very well-suited for this work.

The most important feature provided by LCTFs is their excellent image quality, which does not degrade the diffraction-limited imaging performance provided by high quality optical systems. LCTFs can be readily combined with imaging optics and high fidelity focal plane array detectors as integral components of high definition chemical imaging systems. Figure 2 is an image of a lupus pathology sample taken with a broadband (35 nm FWHM) LCTF placed between the microscope and a 1024^2 element Kodak Megaplus CCD camera. The image was acquired by taking three monochrome images sequentially, in the blue, green, and red wavelength ranges, which were used to print a 24-bit true-color image. This figure illustrates the unparalleled imaging capability of the LCTF.
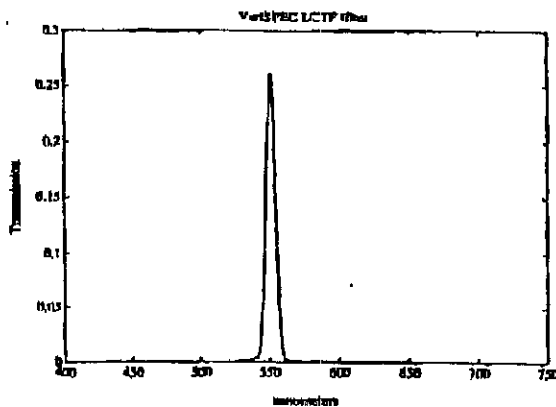
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Fig 1.   Bandpass of an LCTF with   Fig 2.   High-resolution image
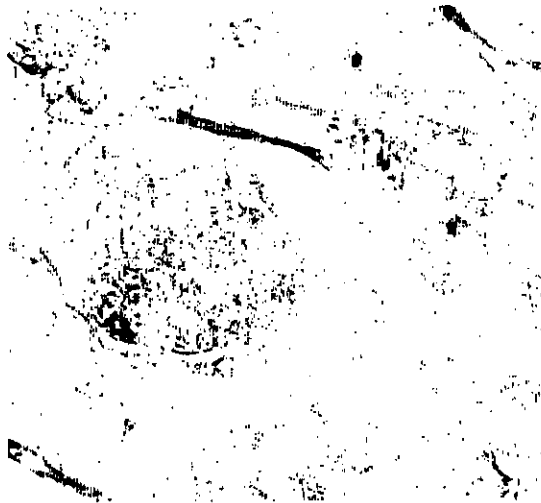10 nm FWHM, tuned to 550 nm.   of lupus sample, through LCTF.

A filter with considerably narrower FWHM is contemplated for the present work.
Off-the-shelf LCTF's have bandwidths ranging from 5 to 50 nm, and tuning
ranges from 400 - 1100 nm.   Specialized devices have been made with 0.2 nm
passband.   The bandwidth of the filter depends only upon the thickness of the
fixed crystal retarder elements employed, and manufacture of high-resolution
filters does not place more stringent demands on the liquid crystal devices,
than when making a spectrally broad filter.   A filter with bandwidth of 0.25
nm or less (10 cm^{-1}) would have broad utility for Raman imaging, and
construction of such a filter is within the present art.   The main technical
challenge in making a narrow bandpass device is to maintain high transmission:
more elements are required, and the optical loss must be minimized.   While
light throughput is important, out-of-band rejection is just as important
because of the need for discrimination between the Raman band of interest and
potential interferences including fluorescence, Rayleigh scattering, and other
Raman bands.

E.2   Related Research
Several approaches to Raman microscopy have been developed, including rotating
dielectric filter tuning [6], laser scanning [7], spatial multiplexing [8],
and tunable excitation with fixed bandpass filtering [9]; however, the last
three are not believed to be commercially competitive with the high-resolution
imaging discussed in this proposal.

At present there are three viable alternative methods to the LCTF for high-
definition Raman imaging, based on rotating dielectric filters, acousto-optic
tunable filters (AOTF) [10-11], and liquid crystal etalon (LCE) technologies
[12].   The rotating-interference filter approach employs moving mechanical
parts to tilt dielectric interference filters, and thus effect wavelength
tuning of the Raman emission collected through a microscope.   The imaging
quality is good at near-normal incidence angles, but upon rotation of the
filter the Raman images shift.   In practice, the collection of complete Raman

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spectral image sets is time-consuming and suffers from spectral artifacts.
The technology does not compare favorably with electronically-tunable filters.

In an AOTF, an RF sound wave generated by a piezoelectric transducer passes
through a birefringent crystal, establishing a transmission diffraction
grating within the crystal. Light meeting a wavelength-dependent momentum
condition interacts with the grating and changes its angle or polarization
state. The AOTF chief advantages are their tuning speed (25 µs) and high
throughput (40%). Speed is irrelevant in Raman imaging applications: an
imaging Raman spectrometer will dwell at each wavelength for a second or more,
so microsecond tuning is not needed. Apertures are limited, from 2 to 10 mm
square, and the devices accept only highly collimated light. They exhibit
relatively strong sidelobes outside the passband, which can be reduced to
approximately 3 percent by apodization of the RF signal.

AOTF's have recently been applied to Raman microscopy [10-11]. Because they
operate on a diffraction principle, there is an image shift as the AOTF is
tuned. This causes problems in multispectral imaging, as precise image-to-
image registration cannot readily be achieved without optical redesign of the
AOTF, incorporation of compensation optics, or software correction. Further,
since passband sidelobes have a different wavelength from the passband center,
they are diffracted at different angles, which results in spectral 'smearing'
and degraded image resolution. In a side-by-side comparison at 647.1 nm it
was observed that use of an AOTF limited the resolution of a microscope image
to ~1 µm, while the LCTF maintained the diffraction-limited resolution of the
microscope [13].

Like an LCTF, an AOTF operates on one linear polarization at a time. As a
result of this, and internal losses, the maximum throughput is between 40 and
45 percent for highly collimated light. Commercially available imaging AOTF's
have bandpasses of 2 nm at 633nm (50 cm^{-1}), several times what is desired.
Attaining bandwidth of 10 cm^{-1} may be possible, but only in devices with lower
acceptance angle, which effectively reduces the field-of-view seen under the
microscope. The wavelength reproducibility of tuning is sufficient for Raman
spectroscopy. In summary, AOTFs offer superior speed while sacrificing
performance in all other areas: aperture (field-of-view), imaging quality,
spectral resolution, and out-of-band rejection.

Another approach to the tunable spectral filter has been taken by Dr. Michael
Morris, who has used a liquid crystal etalon (LCE) to collect Raman images
[12]. These have liquid crystal material within the cavity of an optical
etalon, and the electro-optic action of the liquid crystal material provides
an adjustable optical index for tuning. Because only the extra-ordinary
optical index n_e is tuned, light incident on the LCE must be linearly
polarized. The free-spectral range of an LCE is typically 5 - 25 nm, and
finesses ranging from 8 - 25 have been reported [14-15] for near-visible
LCE's. Bandwidths as narrow as 0.2 nm should be possible, set by the maximum
practical thickness of the liquid crystal layer. However, transmission drops
off rapidly with increasing finesse, due to cavity losses. These losses are
much higher than in dielectric interference filters or air-spaced etalons, due
to scatter in the liquid crystal material and absorption in the alignment and
electrode layers [16]. The spectrum of a typical commercial etalon [17] from

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Meadowlark Optics is shown in Figure 3A, showing finesse of 8.5 and out-of-band blocking of 36:1 at 570 nm.
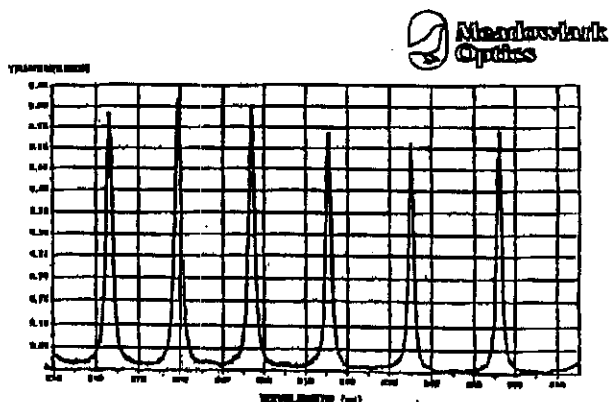
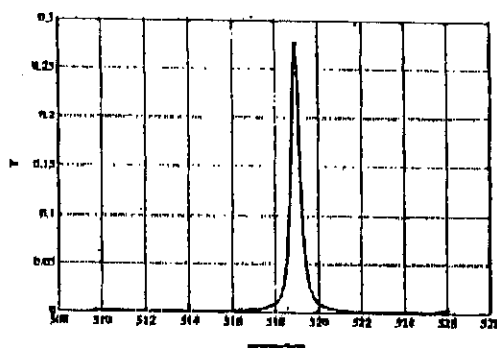

Fig 3A.   Bandpass of a commercial LCE device.

Fig 3B.   Bandpass of high-performance LCE made by P.I.

The Principal Investigator has constructed what are believed to be the highest-performance LCE devices to date. A spectrum from one is shown in Figure 3B, exhibiting a finesse of 21, and out-of-band blocking of 204:1. Other devices for infra-red use showed finesse of 53 and blocking of 450:1. But even these high-performance devices have limitations which make them unsuitable for Raman imaging applications. First, while they are mechanically stable, they have great thermal instability. The optical index of liquid crystals has a thermal coefficient 240 times that of quartz [18-19], so there is a large drift, in addition to drift in the electro-optic tuning action. Another source of drift is the thermal coefficient of expansion of liquid crystal fluid, roughly 800 times that of glass. Since liquid crystal material is essentially incompressible, temperature changes cause great pressures within the sealed liquid crystal cavity [20]. This distorts the etalon figure; changing its pass wavelength and degrading its finesse. For these

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reasons, their pass wavelength is not stable over time and temperature, and any practical LCE system requires a daily *in situ* wavelength calibration.

Second, LCEs offer limited throughput. While the peak transmission of a low-finesse etalon can be high (up to 60% for polarized light), generally two or more of LCEs are needed in order to obtain sufficient tuning range and high overall finesse. This reduces the transmission and greatly increases the complexity: the two LCE elements must be made to tune together, although each device has substantial tuning drift. Another limit is the field-of-view. The field-of-view is different from that of a simple Fabry-Perot etalon, because the LCE cavity is filled with anisotropic crystalline material that is electro-optically flexed, and exhibits a wide range of orientations at different points throughout the cavity. No analytical expression for the field-of-view has been reported, but measurements suggest that an f/32 or slower beam is required for a 15 cm^{-1} device, corresponding to 2° field-of-view [16]. Since the peak transmittance and field-of-view of practical LCE systems are equal to or lower than LCTF figures, throughput will necessarily be lower.

In summary, the imaging quality of LCEs is high, but even the best devices have substantial spectral leakage. A minimum of 10^3:1 contrast is required to gather high fidelity spectral images, which is beyond the capability of present devices. At least two LCE devices must be ganged together to yield sufficient resolution and free-spectral range, which reduces transmission and adds to system complexity because of tuning difficulties in these parts. There are significant problems with thermal sensitivity and wavelength accuracy, and field-of-view is much less than for LCTF devices.

E.3 Innovativeness and Originality of the Proposed Research
High-performance LCTFs were first demonstrated by the C.R.I. researchers [21-22], and the P.I. holds two patents relating to their construction and tuning [23-24]. C.R.I. has won three industry awards for the VariSPEC filter, based on this technology, and it is presently the only firm supplying scientific-grade LCTF filters.
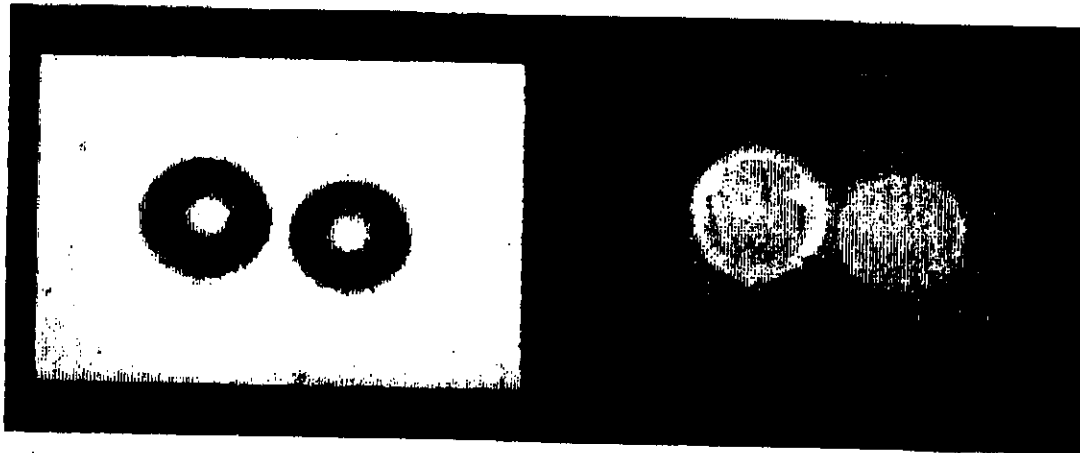

Fig. 4A. Brightfield image of   Fig. 4B. Raman image of the same
45μm test microspheres.   45 μm spheres, taken with LCTF.

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The first use of liquid crystal tunable filters for Raman microscopy was
performed at the University of Pittsburgh in a collaborative effort between
C.R.I. researchers and Prof. Treado [13]. Figure 4A is a brightfield image of
a ~45 μm diameter polystyrene microsphere that has not been tagged with a
fluorophore. Figure 4B is a Raman image of the microsphere collected in 10
secs. The LCTF passband is centered at 691.5 nm which corresponds to a filter
position of 992 cm^{-1}, the relatively intense ring deformation band of
polystyrene. The image quality demonstrated in Fig. 4B surpasses the imaging
performance of any of the alternative Raman imaging methodologies [6-9].

The diffraction-limited imaging performance of the LCTF combined with its
tunability is unparalleled in Raman imaging. The imaging performance combined
with the demonstrated reproducible spectral tuning of LCTFs predict that the
technology proposed here will revolutionize the practice of Raman imaging.

F.   Phase I Research Objectives

F.1   Specific Phase I Objectives
The Phase I Technical Objectives are:

1)   To use existing computer models to generate a tunable Lyot filter design
      optimized for Raman spectroscopy;

2)   To study the integration of such a filter into a microscope imaging
      system, including optical path, data acquisition, and camera
      requirements;

3)   To construct a fully imaging prototype filter according to this design,
      with resolution of 10 cm^{-1} or better, tunable over a range of 2500 cm^{-1};

4)   To characterize this filter for its spectral properties, including peak
      transmission, bandwidth, out-of-band rejection, and tuning range;

5)   To use this filter to obtain true two-dimensional Raman spectroscopy
      images of test samples; and,

6)   To prepare a Phase I report describing the results.

F.2   Connection with Phases II and III
Phase I of the anticipated program will demonstrate feasibility by integrating
a high resolution LCTF filter into a microscope, and obtaining Raman images of
various chemical test samples. Phase II will involve further optimization of
the high definition LCTF filter, and development within an integrated, turnkey
Raman microscope consisting of a laser, microscope, LCTF, and CCD detector,
with processing and analysis software. Microscope integration will be
performed in collaboration with ChemIcon, Inc. a start-up company located in
Pittsburgh, PA.

In Phase III, ChemIcon will perform the system integration and market these
microscopes to existing Raman microspectroscopy customers of ChemIcon,
including the six Fortune 500 companies which currently support the

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development of Raman microscopy in Prof. Treado's laboratory. Raman microscopy is being applied to analyze a host of materials relevant to the specific industrial sponsors. Size of the mature market is estimated as several hundred microscopy sites, with annual sales of $5M or more.

G.   Phase I Research Plan

G.1   To design a tunable Lyot filter optimized for Raman spectroscopy
Over the past 5 years, the P.I. and co-workers at C.R.I. have developed computer models for the design of Lyot filters, based on the Jones calculus and incorporating spectrally-dependent values for the transmission, absorption, and birefringence of the materials involved. These include routines for calculating response for off-axis rays, and the tuning error of the liquid crystal retarders, based on actual measured statistical variation of these components from ongoing production lots. These models have a proven ability to predict passband width, spectral leakage, and peak transmission.

The principal requirements for a Raman-optimized LCTF are narrow bandwidth, high transmission, low leakage, and wide field-of-view. The tuning range will be 515 - 750 nm, to permit use with various laser sources ranging from 514 nm (Argon) to 647 nm (Krypton). A filter with bandwidth of 0.25 nm at 500 nm (10 cm^{-1}) requires use of calcite or other high-birefringence materials for the fixed retarders. This, coupled with the wide field-of-view requirement, leads to a design based on wide-field elements [25-26]. It is likely that $LiNbO_3$ will be chosen as the retarder material, rather than calcite, because of its lower δn value. Consequently, its figuring error tolerances are relaxed relative to those of calcite. Also, it is more rugged, and not as prone to chipping and scratching.

High transmission in the filter is a given by $T - E^s$, where s is the number of Lyot stages and E is the efficiency per stage. The stage efficiency E is set by losses in the liquid crystal cell, the polarizer, the fixed retarder, and tuning errors. These are input parameters to the design, based on measured values. In the design process, one seeks to minimize the number of stages, s, while meeting the requirements for bandwidth, free-spectral range, and out-of-band blocking. While the classical Lyot design offers little latitude in this regard, such a design is rarely suitable for a tunable filter. This is because the exact value of retardance at each stage will deviate from the idealized binary sequence Lyot described, as it is tuned to various wavelengths. To avoid sidelobes in the resultant filter response, one must add a few additional stages for contrast enhancement. The selection of retardances for these stages plays a crucial role in determining filter performance, and proper choice allows their number to be minimized.

The figure of $s - \log_2(FSR/FWHM)$ for a classical Lyot design, represents a lower limit for an LCTF design. For a Raman filter, broad pre-filters can be used to define a useful range of 50 nm, so this is an acceptable FSR. This, coupled with the bandwidth FWHM of 0.25 nm, leads to a lower limit of s=8. A practical LCTF filter will probably require 3 or more additional stages, or a total of 11.

In addition to the optical components, we will design a suitable mechanical enclosure to provide a stress-free filter mounting. The thermal management approach is to create a nearly isothermal environment, free of gradients across the filter, and to sense the temperature with a medical-grade chip thermistor. This allows monitoring temperature with 0.05 °C precision, which is sufficient to resolve spectral shifts as small as $\delta\lambda \approx .01$ nm in the fixed retarders, corresponding to 1/25 of the filter FWHM. Unlike fixed-wavelength Lyot filters, there is no need to stabilize the filter temperature: the liquid crystal elements will be used to actively compensate for thermal drift in the $LiNbO_3$ and quartz retarders [24].

G.2   To study integration of an LCTF into a microscope imaging system
Integration of the LCTF into a Raman imaging system will involve both opto-mechanical and software engineering. Key C.R.I. researcher Clifford Hoyt has considerable experience integrating LCTFs in microscopes for fluorescence and pathology applications. Approaches have included placing the LCTF in the infinity-corrected optics path of microscopes such as Zeiss Axioskop and Olympus B-MAX systems, as well as the use of relay optics and telecentric correction systems. The Raman imaging LCTF will have a 20 mm aperture, and an overall length of 40-55 mm, similar to that of LCTF filters previously used for fluorescence work. It is anticipated that Prof. Treado will perform computer-aided optical design, using Zemax XE ray tracing software, to model the integration of the LCTF into microscopes in his laboratory. The location of other strategic components of the Raman system can also be modeled and optimized using this technique.

For the software integration, Prof. Treado will define the necessary features of Windows computer program to control the LCTF and to perform sophisticated multi-spectral image processing. A list of features and functions will be developed for actual software coding in Phase II of the project. Existing software allows Prof. Treado to acquire, display, and store high-resolution Raman images, and to control the filter manually for the Phase I testing.

G.3   To construct a fully imaging prototype filter according to this design
C.R.I. routinely manufactures commercial LCTF's for use in this spectral range, with bandwidths of 5-35 nm. We do not anticipate making any changes to the liquid crystal tuning elements for the present work.

The only special concern we anticipate in assembling this filter relates to reflections at the $LiNbO_3$ retarders. LCTF filters are normally assembled in an index-matching material which keeps reflections below 0.03% per interface. However, $LiNbO_3$ has a substantially higher optical index than the other materials used (n_o=2.30 vs. n=1.53), so it needs to be anti-reflection coated to match its impedance to that of the index-matching material. From the analysis of Staromlynska, inter-reflections limit the extinction of each stage to $1 / [3R^2(1-R)]$, including the effects within the liquid crystal cell [27]. For the coated $LiNbO_3$ interfaces, a reflection R of 0.5% or less is anticipated, yielding a contrast of 13,400:1 per stage. Since LCTF designs require a contrast of only 100:1 per stage, there should be no problem meeting project goals for the overall filter contrast.

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Electronics modules for tuning the liquid crystal cells and sensing the
thermistor resistance have been developed in the course of commercial LCTF
development, and will be used without modification in the present work.

G.4   To characterize this filter for its spectral properties
The filter will be characterized at C.R.I. using a SPEX 0.5M spectrometer with
0.02 nm resolution. Bandwidth (FWHM) and peak transmission will be measured
using a quartz-halogen lamp source, and a Hammamatsu S1336 detector with a
Scitec chopper and SRS 810 lock-in amplifier. This provides measurements of
peak transmission that are accurate to 2% of the transmission value, and
resolves transmission of 10^{-5} or less, for spectral leakage measurements.
Tuning range will be checked directly, by observing the filter response as it
is tuned across the operating range. Measurements are fully automated using a
PC/486 controller, enabling hundreds of spectra to be taken with no operator
involvement. Spectral tuning accuracy will be checked at the 543.5 and 632.89
nm laser lines using He-Ne sources.

Off-axis response will be measured by mounting the filter on a two-dimensional
rotation stage and observing passband shift as the LCTF orientation is varied.
Thermal drift will be measured if time permits, by mounting the filter on a
Neslab RTD-110 controlled temperature stage, and measuring passband variations
as the LCTF temperature is adjusted over the range 20 - 35 °C.

G.5   To use this filter to obtain two-dimensional Raman spectroscopy images
The LCTF filter will be integrated into microscopes at the University of
Pittsburgh for these tests, which will be performed by Prof. Treado. Spectral
imaging will be evaluated by measurement of the modulation transfer function
(MTF), a quantitative analysis of imaging performance. The MTF will be
measured by imaging several standard resolution targets as a function of
wavelength, using a Princeton Instruments CCD detector and BioScan OPTIMAS
software to acquire the images. Raman imaging performance will be established
by evaluation of images of polymer microspheres, routinely used as size and
chemical standards by researchers in this field.

Raman imaging evaluation will also be performed on real-world, industrial
materials available to Prof. Treado through collaborations with industrial
sponsors. Materials will include corrosion-resistant alloys, polymer
composites and pharmaceuticals.

G.6   To prepare a Phase I report describing the results.
The P.I., Prof. Treado, and senior C.R.I. personnel will draft a Phase I Final
Report for submission to the NSF, including experimental data, images, and
technical analysis, in accordance with N.S.F. guidelines.

H.   Commercial Potential

H.1   Brief Description of the Company
Cambridge Research and Instrumentation, Inc. was formed in May 1985 with the
aims of carrying out research in solar-terrestrial physics and developing

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instruments for the accurate measurement and control of light. Dr. Peter
Foukal, president of C.R.I., Inc. has published over 100 papers in major
refereed journals and conference proceedings. This work on solar astrophysics
has also been presented in numerous contributed and invited papers at meetings
of the AAS, AGU and IAU. Dr. Foukal has also served on various advisory
committees and panels of NASA, NSF, and the National Academy of Sciences, over
the past fifteen years. His text book "Solar Astrophysics" was published by
Wiley Interscience in May 1990.

C.R.I. maintains active programs at the forefront of solar-terrestrial
research, including photometric and radiometric studies of solar irradiance
variation, and also remote sensing studies of plasma electric fields in the
sun's atmosphere. Much of this work is carried out at the National Solar
Observatories stations at Kitt Peak and at Sacramento Peak, and has been
supported by the NSF, NASA, and the Air Force.

In electro-optics, C.R.I. has developed the LaseRad and CryoRad cryogenic
cavity radiometers as laboratory standards for measurement of radiative flux,
with support from the SBIR Programs of the NSF and NASA. These instruments
are in use as national standards of light measurement at NIST (ex-NBS), and in
Germany, Sweden, Canada, Spain, and at the CNAM in France. C.R.I. also
developed and builds commercially the award-winning LS and LPC series laser
power stabilizers widely used in electro-optics laboratories, and most
recently, the award-winning VariSpec liquid crystal tunable filters. A
significant fraction of C.R.I.'s $1.2M sales volume in the last fiscal year
consisted of exports of commercial products to Europe and Japan.

H.2   Commercial Applications
H.2.a Polymer Imaging
Raman vibrational spectroscopic imaging of heterogeneous polymer dispersions
and polymer thin films will be possible when the LCTF filtering technology is
suitably incorporated into a dedicated Raman microscope. Such a device will
have broad applicability in the polymer and coatings industries because it
will allow for non-invasive, chemically specific visualization of domains and
defects in polymer composites without the need for sample staining.

At present, many analytical imaging methodologies are applied to polymer
characterization. The optical microscope is employed in combination with a
host of optical contrast generation mechanisms including polarization,
interference and fluorescence. In general, these methods do not provide
chemical specificity and provide limited spatial resolution. For high spatial
resolution analysis, polymers are imaged with electron microscopy, usually in
combination with osmium tetroxide staining, a difficult and hazardous
undertaking. Existing polymer visualization methodologies are problematic.
Raman imaging has extraordinary potential in polymer analysis but the power of
the technique hinges on the availability of a reliable, high resolution Raman
spectral filter.

Prof. Treado is collaborating with a major pharmaceutical firm and Ford Motor
Co. to develop Raman imaging instrumentation suitable for characterizing the
polymeric materials of importance to these companies. If the suitable

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filtering technology can be developed these industrial sponsors will be a
ready market for Raman microscopes.

H.2.b Corrosion Imaging

Non-invasive, *in situ* monitoring of temperature-dependent corrosion of steel
alloys exposed to phosphate solution is an important application of Raman
imaging. This will be performed with long working distance microscope optics
to probe samples housed in a programmable high temperature stage, viewing them
through protective diamond windows. A rapid and non-invasive method for
monitoring corrosion *in situ* provides several capabilities: first, chemical
mechanisms of corrosion can be established; second, failure rate predictions
that dictate the replacement schedule of strategic materials can be
determined, representing a tremendous potential cost saving to industry and
government; and, methods to inhibit corrosion can more effectively be
evaluated. Methodologies developed here will be generally applicable to any
in situ Raman imaging application where samples are housed in extreme
environments of temperature, pressure, or radiation.

C.R.I. will develop high resolution LCTF technology to be coupled with
microscope optics and CCD cameras to provide submicron spatial resolution with
sufficient spectral resolution to distinguish the corrosion chemical species.
The P.I. and Prof Treado will work with industrial sponsors interested in
monitoring *in situ* temperature-dependent corrosion of alloys. At present,
Prof. Treado is providing a customized microscope to two Fortune 100 defense
contractors who perform these types of measurements under contract to the
Department of the Navy. The technologies developed in these programs will be
directly applicable for corrosion monitoring aboard Navy ships, and in steam
and nuclear power generators. Once Raman corrosion monitoring technology is
developed it will be marketed to the commercial power industry.

H.2.c Crystal Polymorphism Imaging

Raman microscopy has the potential for use in quantitative polymorphism
characterization of pharmaceutical active-agent crystalline material.
Polymorphism occurs in pharmaceuticals in which a chemical constituent may
have more than one crystalline structure. This is problematic because an
active agent may provide therapeutic benefit only if it is the appropriate
crystalline form or polymorph. Raman microscopy can often distinguish
spectroscopically between different polymorphs, and Prof. Treado is engaged in
a collaboration with a large international pharmaceutical firm to establish
the utility of Raman microscopy as a quantitative methodology for
discriminating different polymorphs in a quality control application. If
feasibility can be established a market for Raman microscopes will be defined.
Raman systems relying on LCTFs will then be marketed to pharmaceutical
companies for polymorphism quantitation.

H.2.d Biomedical Imaging

Probing biological structures is tractable with Raman spectroscopy. In
particular, Raman spectroscopy is effective for analyzing aqueous samples
without the interference of the strong OH bands encountered in infrared
spectroscopy. In addition, the intrinsic vibrational markers of biological

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samples provide chemical selectivity without the need for significant sample preparation or the use of potentially invasive stains or tags. In macroscopic, non-imaging studies of biological materials, the wealth of chemical information provided by Raman spectroscopy is often convoluted due to the complexity of the materials. Raman spectroscopic microscopy focuses the chemical analysis to localized regions of heterogeneous materials, and provides an efficient chemical visualization method. Biological applications of Raman microscopy include resonance Raman studies of carotenoid pigments of bacteria [28], Raman microspectroscopy of gallstones and kidney stones [29], and of DNA in single tumor cells [30].

H.3   Competitive Products

In 1991 SPEX licensed the spatial multiplexing approach co-developed by Prof. Treado at the University of Michigan, but the rapid pace of development in Raman microscopy makes the spatial multiplexing approach obsolete.

In the U.K., Renishaw P.L.C. commercialized a dedicated Raman microscope employing interference filter tuning. The microscope combines Raman imaging optics with a microprobe. In operation, Raman microspectra are collected rapidly at isolated regions of a sample, and then full-frame images are collected with the filters tuned to a few discrete Raman bands. Complete spatial/spectral information cannot readily be collected with this approach. It is well suited to visualization of widely-separated Raman bands, but not to imaging of dense matrices in which complex Raman spectra often overlap and are convoluted. To obtain a definitive evaluation of complex systems, the kind of materials encountered in the real world, the spectral collection and image collection must be integrated, and provide the kind of complete spatial/spectral data available from a tunable imaging spectrometer.

Approaches requiring the use of moving mechanical parts contribute to degraded imaging performance. The imaging performance is especially compromised when widely separate Raman spectral ranges are accessed.

A no-moving-parts approach to Raman microscopy has been successfully demonstrated by Prof. Treado and co-workers at the National Institutes of Health employing AOTFs [7]. Despite the no-moving-parts design, the AOTF imaging quality is handicapped due to the 'spectral smearing' described earlier in Section E.2. Future AOTFs may address these degradations, and may provide the high spectral resolution (10 cm-1), and large optical apertures (~ 25 mm), anticipated in the proposed technology, but current AOTFs do not. As importantly, the development of optimized AOTFs is much less tractable than the development of LCTF technology. High resolution LCTFs have already been fabricated for other spectroscopic applications and can be optimized readily for Raman microscopy.

AOTF microscopes are currently being commercialized by Brimrose Corp. of America. These devices are not manufactured under license with NIH, which has a patent pending on the technology. If the patent is issued the Brimrose microscopes will infringe on the NIH patent and it is anticipated that the federal government will defend their patent rights.

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It is anticipated that the Renishaw Raman microscope is the competitive
product for the foreseeable future.

H.4   Advantages of the Proposed Approach Over Existing Technology
The existing competitive technologies are tunable spectral filter designs
including rotating dielectric filters, AOTFs and LCEs. The LCTF approach
enjoys a competitive advantage over all of these alternatives.

The complete spatial/spectral data generated by LCTF system gives it a clear
advantage over rotating dielectric filter systems, because it provides the
user with a much more detailed picture of the chemical distribution when
characterizing spatially and spectrally complex, real-world samples.

Compared to systems using AOTF filters, an LCTF-based Raman imaging system
would offer vastly superior imaging quality. In addition, use of a liquid
crystal waveplate at the LCTF entrance enables monitoring the polarization
state of the Raman signal. This yields important benefits for studies of
oriented anisotropic materials such as crystals, polymers, semiconductors, and
fibers. System integration is easier as well, due to the larger aperture and
viewing angle the LCTF affords. Turning to LCEs, if these are commercialized
in the future, they promise severe limits in terms of spectral calibration,
out-of-band blocking, and field-of-view, without offsetting performance
benefits in other areas. This suggests that the LCTF will enjoy a competitive
advantage over this technology as well.

H.5   Progress in Commercializing Other SBIR Technology
The Principal Investigator has developed several innovative instruments for
optical research.   He was one of the key members of the team that pioneered
the use of cryogenic cavity radiometer for laboratory standards measurements
of radiative flux, with support from the Small Business Innovation Research
Programs of the NSF and NASA. These instruments are in use as national
standards of light measurement at NIST (ex-NBS), the German PTB, CNAM in
France, as well as the national laboratories of Sweden, Spain, and Canada.
C.R.I. also developed and builds commercially the award-winning LS and LPC
series laser power stabilizers used worldwide in electro-optics laboratories,
and most recently, the patented VariSpec line of liquid crystal tunable
filters. The VariSPEC was supported by NSF and NIH Small Business Innovation
Research Programs. These instruments, with over $2 million in commercial
sales, point to a long and successful history both in research and in the
development and marketing of scientific research equipment.

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I.   Principal Investigator and Senior Personnel

PETER J. MILLER   PRINCIPAL INVESTIGATOR
Staff Scientist
Cambridge Research and Instrumentation, Inc.

Education:

    1980 B.A. (Astronomy)   Williams College
    1985 M.S. (Electrical Engineering) Dartmouth College

Memberships:

    American Astronomical Society
    Optical Society of America

Experience:

1979   Visiting Fellow, JILA/NBS, Boulder, CO
1980-83   Staff Scientist, Atmospheric and Environmental Research, Inc.,
            Cambridge, MA
1985   Staff Scientist; Cambridge Research and Instrumentation, Inc.,
            Cambridge, MA

Relevant Publications, Reports, and Awards

    "Optical Retarder Having Means for Determining the Retardance of the
    Cell Corresponding to the Sensed Capacitance Thereof", U.S. Patent
    5,247,378 (1993).

    Laser Focus CTA award, Laser Focus, 29, 115 (1993). Awarded for the
    VariSpec tunable liquid crystal filter.

    R & D 100 Winners Announcement, Research & Development, 34, 12 (1992).
    Awarded for the VariSpec tunable liquid crystal filter.

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    (1992). Awarded for the VariSpec tunable liquid crystal filter.

    "Use of Tunable Liquid Crystal Filters to link Photometric and Radiomet-
    ric Standards", P. Miller, Metrologia 28, 145, (1991).

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    Miller, SPIE Proc. 1235, 466, (1990).

    "Liquid Crystal Devices and Systems Using Such Devices." P. Miller,
    U.S. Patent 4,848,877 (1989).

    "Tunable Birefringent Filters Using Liquid Crystals", P. Miller, talk
    presented at "Optics for Astronomy and Remote Sensing", O.S.A. Topical
    Meeting, Sept. 26-29, 1988, Falmouth, MA.

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CLIFFORD C. HOYT
Staff Scientist
Cambridge Research and Instrumentation, Inc.

<u>Education</u>
     1983 B.A. (Physics) Williams College
     1987 M.S. (Engineering) M.I.T.

<u>Experience</u>

     1983-85   Staff Scientist, Atmospheric and Environmental Research,
                Inc., Cambridge, MA
     1987-   Staff Scientist, Cambridge Research and Instrumentation,
                Inc., Cambridge, MA

<u>Relevant Publications</u>

"Imaging Spectrometers for Fluorescence and Raman Microscopy:
Acousto-Optic and Liquid Crystal Tunable Filters", Hannah R. Morris,
Clifford C. Hoyt, and Patrick J. Treado, Appl. Spectrosc. 48, (1994) in
press.

"Merging Spectroscopy and Digital Imaging Enhances Cell Research", C.
Hoyt and D. Benson, Photonics Spectra, 26, 92, (1992).

"Characterization of an absolute cryogenic radiometer as a standard
detector for radiant-power measurements", R.U. Datla, K. Stock, A.C.
Parr, C.C. Hoyt, P.J. Miller, and P.V. Foukal, Appl. Opt., 31:34, 7219-
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"Cryogenic Radiometers and their Application to Metrology", C.C. Hoyt,
P.V. Foukal, Metrologia, 28:3, 163-168 (1991).

"Liquid Crystal Tunable Filters for Photometry", C. Hoyt, P. Miller,
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"Image-Preserving Tunable Filter for Microscopy", C. Hoyt, Phase I SBIR
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"Comparison Between a Side-Viewing Cryogenic Radiometer and Self-
calibrated Silicon Photodiodes", C. Hoyt, P. Miller, P. Foukal, E.
Zalewski, SPIE Proc 1109, (1989).

"Remote Biomedical Spectroscopic Imaging of Human Artery Wall." Lasers
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"Spectroscopic Diagnosis for Control of Laser Treatment of
Atherosclerosis." R.R. Richards-Kortum, A. Mahta, T. Kolubayev, C.
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J.   Consultants and Subcontracts

Patrick J. Treado
Assistant Professor of Chemistry
Department of Chemistry, University of Pittsburgh

Prof. Treado is a recognized expert in the development of Raman chemical imaging, optical microspectroscopic techniques and their application to materials analysis. His letter confirming availability and commitment to this project is included as an Appendix to this Proposal.

Prof. Treado performed his doctoral work under the direction of Prof. Michael D. Morris. His research involved the development of spectroscopic imaging techniques, specifically Raman microscopy and photothermal deflection densitometry, employing Hadamard transform spatial multiplexing. The Raman microscopy technique led to several nationally competitive fellowships and awards being won by Prof. Treado. A patent of the Hadamard Raman microscopy was issued in 1991, and the technology has been licensed by Spex Industries, a spectroscopic instrument manufacturer, for commercial development.

While a postdoctoral fellow at the National Institutes of Health (1990-1992) Prof. Treado performed biophysical studies of model biological membrane assemblies using Raman and infrared spectroscopy. With Dr. Neil Lewis and Dr. Ira Levin, Prof. Treado pioneered the development of chemical imaging methods using acousto-optic tunable filters (AOTFs). Prototype instruments capable of performing visible/near-infrared absorption microscopy and Raman microscopy were developed. A patent covering the use of AOTFs in spectroscopic imaging instruments is pending. His paper describing the first use of AOTF for Raman chemical imaging was awarded the 1993 Meggers Award by the Society for Applied Spectroscopy for the best paper published in Applied Spectroscopy in 1992.

Since joining the University of Pittsburgh, Department of Chemistry as an Assistant Professor in September 1992, Prof. Treado has been engaged in a research program in analytical spectroscopy and chemical imaging, including the first use of an LCTF for Raman imaging, with the P.I. of this proposal.

Selected Publications
1. Hannah R. Morris, Clifford C. Hoyt, and Patrick J. Treado, "Imaging Spectrometers for Fluorescence and Raman Microscopy: Acousto-Optic and Liquid Crystal Tunable Filters", Appl. Spectrosc. 48, (1994) in press.
2. Patrick J. Treado, Ira W. Levin, and E. Neil Lewis, "Indium Antimonide (InSb) Focal Plane Array (FPA) Detection for Near-Infrared Imaging Microscopy", Appl. Spectrosc. 48, (1994) in press.
3. E. Neil Lewis, Patrick J. Treado, and Ira W. Levin, "Near-Infrared and Raman Spectroscopic Imaging", Amer. Lab. in press.
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6. Patrick J. Treado and Michael D. Morris, "Infrared and Raman Spectroscopic Imaging, Spectroscopic and Microscopic Imaging of the Chemical State", M.D. Morris, Ed. (Marcell Dekker, New York, 1992) pp. 71-108.

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7. Patrick J. Treado, Ira W. Levin and E. Neil Lewis, "High-Fidelity Raman Imaging Spectrometry: A Rapid Method Using an Acousto-optic Tunable Filter", Appl. Spectrosc. 46, (1992) 1211.
8. Patrick J. Treado, Ira W. Levin and E. Neil Lewis, "Near-Infrared Acousto-Optic Filtered Spectroscopic Microscopy: A Solid State Approach to Chemical Imaging", Appl. Spectrosc 46, (1992) 553.
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11. Patrick J. Treado and Michael D. Morris, "Hadamard Transform Spectroscopy and Imaging", Spectrochimica Acta Rev. 13, (1990) 355.
12. Patrick J. Treado and Michael D. Morris, "A Thousand Points of Light: the Hadamard Transform in Chemical Analysis and Instrumentation", Anal. Chem. 61, (1989) 723A.
13. Patrick J. Treado and Michael D. Morris, "A Hadamard Transform Raman Microprobe", Appl. Spectrosc. 43, (1989) 190.
14. Patrick J. Treado and Michael D. Morris, "Hadamard Transform Raman Imaging", Appl. Spectrosc. 42, (1988) 897.
15. Patrick J. Treado and Michael D. Morris, "A Hadamard Transform Raman Microscope", in Proc. of the International Laser Symposium IV, R.G. Lerner, Ed. (American Institute of Physics, New York, 1989) 725.
16. Michael D. Morris and Patrick J. Treado, "Hadamard Transform Raman Microscopy", in Microbeam Analysis-1989, P.E. Russell, Ed. (San Francisco Press, San Francisco, 1989) 146.

K. Equipment, Instrumentation, Computers, and Facilities

K.1 Facilities available at Cambridge Research & Instrumentation

An equipment item of particular importance to this project is the C.R.I. liquid crystal filter fabrication facility. This facility includes an 8 x 12 foot class-100 clean room, with complete thermal and humidity control; all necessary equipment for fabricating liquid crystal elements of the highest optical quality on glass and quartz substrates; water filtration, substrate handling, and desiccant storage facilities for cleaning and processing to exacting chemical levels; and an 8' Laminaire horizontal flow hood for overall assembly in a particle-free, laminar flow environment.

Test equipment at C.R.I. includes a 0.5-meter SPEX spectrometer with 300 and 1200 line/mm gratings for high-resolution spectroscopy (0.025 nm); a quarter-meter visible ISA spectrometer with an EG&G 1024-element reticon detector; an f/4.5 quarter-meter PTI monochromator, equipped with stepper motors under computer control, with visible and IR gratings; a Scitec optical chopper and Stanford Research 810 lock-in amplifier; quartz-halogen, mercury, Kanthal, and Xe arc continuum sources with collimation optics; a mercury line source for wavelength scale calibration; and a variety of silicon, germanium, PbSe, and InGaAs photodiodes, and photodiode preamps with NIST-traceable calibration.

Other relevant equipment includes a Zeiss Axioskop microscope equipped for epi- and kohler illumination; a Dage MTI CCD-72 camera and controller; a

Matrox MVP-AT frame grabber; Optimus image processing software; Sony
Trinitron RGB monitor; a Neslab RTD-110 controlled temperature system with
heat sink mount for temperature control of optical samples to +/- 0.02 °C;
He-Ne, Ar++, and Nd:YAG lasers with spatial mode filters and beam expanding
optics; 6-inch and 1-inch Labsphere integrating spheres; PC/AT and PC/386
computers with 16-bit high-speed data acquisition hardware and software.

In addition to the above equipment, C.R.I. has a PC/486-based CAD center for
electronic and mechanical design; an electronics test and production lab
equipped to handle analog, digital, and microprocessor assemblies. We have
access to complete metal-working facilities for fabricating jigs, special
optical fixtures, etc. C.R.I. also maintains a NIST-traceable calibration
lab, for detector tests and calibrations at the highest level of accuracy.

K.2   Facilities available to Prof. Treado at the University of Pittsburgh
Laboratory Facilities:
Prof. Treado has approximately 1200 ft^2 of modern laboratory space, equipped
for optical spectroscopy (visible/near-infrared absorption, Raman and
fluorescence emission), optical spectroscopic imaging microscopy, and wet
chemical manipulations.

Computer:
Prof. Treado's laboratory is computer-automated. Data collection is
controlled by personal computers running commercial programs and programs
written by laboratory personnel. Preliminary spectral image data processing
is performed on 486 computers networked to a Silicon Graphics IRIS Indigo R4K
workstation. The workstation functions as a file-server and is connected to a
magneto-optic disk for mass data storage and retrieval. The workstation also
serves as an advanced spectral image processing graphics workstation. It
employs a program, LinkWinds, dedicated to processing and visualizing
multi-dimensional spectral image data sets, which was developed at the NASA
Jet Propulsion Laboratory for remote sensing spectral imaging applications.
LinkWinds is an intuitive and interactive package, allowing unparalleled
capability for multidimensional graphical analysis.

Major equipment in Prof. Treado's laboratory:
(2) Princeton Instruments CCD detectors interfaced to a Gateway 2000 50 MHz
80486 DX2 computer running BioScan OPTIMAS image processing software;
Coherent 330 Krypton ion laser, 400 mW 752 nm, 1 W multiline red; Coherent
CR-3 Argon ion laser, 150 mW 514.5 nm; Brimrose TEAF2-.6-1.1μ acousto-optic
tunable filter; Brimrose TEAF2-1.0-2.0μ acousto-optic tunable filter;
Chromex 0.5 m spectrograph for performing dispersive optical spectroscopy, and
spectral calibration of the LCTFs; (2) Olympus BH-2 upright microscopes with
visible-optimized optics interfaced to a Cohu 4910 b&w video camera for sample
positioning and focusing; Kinetic Systems research grade optical table, 4' x

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10', Newport research grade optical table, 5' x 10'; EG&G PAR 5110 lock-in
amplifier; Electro-Optic Systems InSb photodiode; C.R.I. VariSpec LCTF for
fluorescence imaging.

Special materials:
Sophisticated samples suitable for Raman microscopy applications including
corrosion test samples, polymeric composites, thin films and coatings on
polymer substrates, as well as pharmaceuticals, will be provided by Prof.
Treado's external industrial collaborators in the defense, pharmaceutical, and
automobile industries.

Other:
The chemistry department maintains the usual array of modern spectroscopic
instrumentation available for general access including a scanning electron
microscope, Cambridge Instruments S90. A fully equipped electronic and
machine shop for instrument fabrication and repair is available. Special
purpose equipment can be constructed at J. Baur Machining, Mars, PA, while
most instrumentation repair can be handled on-site.

L.   Current and Pending Support of P.I. and Senior Personnel

The P.I. has no prior, current, or pending support for development of Raman
imaging filters. He is the P.I. of NASA contract NAS7-1246, "Tunable Liquid
Crystal Filters for Remote Sensing Applications", which has a duration of two
years, from May 18, 1993 - May 17, 1995. This carries a 2 month commitment
during the proposed Phase I period of effort.

Clifford Hoyt has been notified of a pending SBIR award from the N.I.H. for a
proposal, "High-Efficiency Tunable Fluorescence Emission Filter" (contract
number not yet available), which begins September 1, 1994 and ends February
28, 1995. He has a commitment of 3 months, 1 month of which falls within the
period of the proposed Phase I effort. In addition, he has been notified of a
pending STTR Phase I award, "A Universal Compensator for Polarization
Microscopy", from the N.I.H. (contract number not yet available), which begins
July 1, 1994 and ends Dec. 31, 1994. Phase II work could begin as early as
May 1995, if a follow-on award is made. Finally, he has a commitment of 20
percent to ongoing commercial projects at C.R.I., amounting to 1.2 months
during the Phase I period.

M.   Equivalent Proposals to Other Federal Agencies
There are no equivalent proposals under consideration by or being submitted to
any other Federal Agencies.

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N.   Budget
The budget is presented on the following page. Funds are budgeted for the
purchase of 10 optical retarder of LiNbO₃ @ $250 each, and $250 for materials
used in making the liquid crystal tuning elements. These supplies will be
used to make the prototype LCTF described in the Plan of Work. The computer
budget is to allow for maintenance and repair costs for the PC/386 systems.

O.   Prior Phase II Awards
Cambridge Research & Instrumentation, Inc., has not received more than 15
Phase II SBIR awards in the past 5 fiscal years.

APPENDIX D

SUMMARY
PROPOSAL BUDGET

ORGANIZATION
...bridge Research & Instrumentation, Inc.

PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR
...er Miller

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION	
	Proposed	Granted
AWARD NO.		

SENIOR PERSONNEL: PI/PD and Other Senior Associates (List each separately with title, A.8, show number in brackets)	NSF Funded Person-mos. CAL.	Funds Requested By Proposer	Funds Granted By NSF (if Different)
Peter J. Miller, P.I.	1.5	$ 6560	$
Clifford C. Hoyt, Staff Scientist	2.0	8024	
( ) OTHERS (LIST INDIVIDUALLY ON BUDGET EXPLANATION PAGE)			
(2) TOTAL SENIOR PERSONNEL (1-5)	3.5	14584	
OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)			
( ) POST DOCTORAL ASSOCIATES			
(1) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	.75	2043	
( ) GRADUATE STUDENTS			
( ) UNDERGRADUATE STUDENTS			
( ) SECRETARIAL - CLERICAL			
( ) OTHER			
TOTAL SALARIES AND WAGES (A+B)		16627	
FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS) .43 X (A+B)		7150	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A+B+C)		23777	
PERMANENT EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $500.)			
(Do not use for Phase I)			
TOTAL PERMANENT EQUIPMENT			
TRAVEL 1. DOMESTIC (INCL. CANADA AND U.S. POSSESSIONS			
2. FOREIGN (Do not use for Phase I)			
PARTICIPANT SUPPORT COSTS			
1. STIPENDS $ _____			
2. TRAVEL _____			
3. SUBSISTENCE _____			
4. OTHER _____			
( ) TOTAL PARTICIPANT COSTS			
OTHER DIRECT COSTS			
1. MATERIALS AND SUPPLIES See proposal text		2750	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION			
3. CONSULTANT SERVICES			
4. COMPUTER (ADPE) SERVICES		200	
5. SUBCONTRACTS Prof. Patrick Treado 35 days@420/day		14700	
6. OTHER			
TOTAL OTHER DIRECT COSTS		41427	
TOTAL DIRECT COSTS (A THROUGH G)			
INDIRECT COSTS (SPECIFY) Overhead .48 X (A+B+C) = 11413			
TOTAL INDIRECT COSTS G & A .155 X (H + Overhead) = 8190		19603	
TOTAL DIRECT AND INDIRECT COSTS (H+I)		61030	
FEE (If requested; maximum equals 7% of J) .065 X J		3970	
TOTAL COST AND FEE (J + K)		$65000	$

PI/PD TYPED NAME & SIGNATURE	DATE	FOR NSF USE ONLY		
Peter Miller _signature_	06/10/94	INDIRECT COST RATE VERIFICATION		
CO. REP. TYPED NAME & SIGNATURE	DATE	Date Checked	Date of Rate Sheet	Initials-DGA
Peter Foukal _signature_	06/10/94			

NSF FORM 1030A (3/94) SBIR

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June 9, 1994

Mr. Peter Miller
Cambridge Research & Instrumentation Inc.
21 Erie Street
Cambridge, Massachusetts 02139

Dear Mr. Miller:

In my work at the University of Pittsburgh, I am pursuing the development of Raman chemical imaging microscopy and its application to industrial process monitoring and the chemical characterization of materials. The current limitation in Raman imaging methodology is the lack of a high resolution tunable filter technology. CRI's track record in liquid crystal tunable filter (LCTF) development and commercialization suggests that the last remaining technological limitation in Raman imaging may soon be resolved. I am committed to aiding the Raman LCTF development process as a consultant and I am providing this letter in support of CRI's Phase I SBIR application to the National Science Foundation entitled "High Definition Raman Imaging Microscope".

My role in the project is to assist in the development of Raman LCTFs and their application to Raman chemical imaging. I will perform the feasibility evaluations that will establish the utility of LCTFs in Raman imaging. My consultant rate is $420/day. After Phase I is successfully demonstrated I will assist CRI in Phase II by developing a prototype Raman imaging microscope system employing high definition LCTFs. In addition, through my involvement with industrial end users of Raman chemical imaging microscopy I will assist in the transfer of the technology to market.

I look forward to CRI's success in LCTF development for Raman imaging.

Sincerely,

Patrick J. Treado
Assistant Professor of Chemistry

Appendix A

Phase I -APPENDIX A

SMALL BUSINESS INNOVATION RESEARCH (SBIR) PHASE I
REPORT COVER SHEET

NSF Award Number: DMI-9560600

Date: September 13, 1996

Grantee Name: Cambridge Research &
                Instrumentation, Inc.
Grantee Address: 21 Erie Street
                Cambridge, MA   02139

Project Title: SBIR PHASE I: High Definition
               Raman Imaging Microscope
Period Covered by This Report: 3/1/96 – 8/31/96

P.I. Name: Peter J. Miller

Please check as appropriate:

☒ Final Report*

* Report content requirements are identified in Article 5 of the SBIR Phase II Grant General Conditions (12/95). This Cover Sheet is required for submission of all reports. Reports should be attached to this Cover Sheet.

☒ Plan to submit Phase II Proposal

☐ Proposal contains Proprietary Information

Certifications:

I certify that the Principal Investigator currently is ☒ , is not ☐ , "primarily employed" by the grantee organization as defined in the SBIR Solicitation.

I certify that the work under this project has ☐, has not ☒, been submitted for funding to another Federal agency and that it has ☐ , has not ☒, been funded under any other Federal grant, contract, or subcontract. .

I certify that to the best of my knowledge the work for which payment is hereby requested was performed in accordance with the award terms and conditions and that payment is due and has not been previously requested.

I certify that to the best of my knowledge (1) the statements herein(excluding scientific hypotheses and scientific opinions) are true and complete, and (2) the text and graphics in this report as well as any accompanying publications or other documents, unless otherwise indicated, are the original work of the signatories or individuals working under their supervision. I understand that the willful provision of false information or concealing a material fact in this reprot or any other communication submitted to NSF is a criminal offense (U.S. Code, Title 18, Section 1001).

Authorized Grantee Representative: _____   Date: 9/13/96

P.I. Signature:_____   Date: 9/13/96

NSF Form 1372 (SBIR 2/96)

Final Report on SBIR Phase I Contract DMI-9560600
"High Definition Raman Imaging Microscope"

submitted to the
National Science Foundation
Arlington, VA 22230

by
Cambridge Research & Instrumentation, Inc.
Cambridge, MA 02139

_____
Principal Investigator

_____
President

9/13/96
_____
Date

9/13/96
_____
Date

Abstract

Technical Summary.

During Phase I, a liquid crystal tunable filter (LCTF) was designed, built, and characterized for use in Raman chemical imaging. The filter has a design bandwidth of 8 cm^{-1} and a tuning range of 500 - 650 nm, or 4600 cm^{-1}. Unlike other tunable elements for Raman imaging, the LCTF is free of optical distortions, spectral leakage, or image shift with tuning.

The actual filter performance met all model predictions, and was successfully integrated into an optical microscope employing a 514.5 nm Ar^+ laser source coupled via fiber-optics to provide uniform illumination through infinity-corrected microscope optics. Raman images were collected using a cooled CCD camera. Sample spectra obtained through the LCTF are identical with spectra of the same samples acquired with a conventional (non-imaging) dispersive spectrometer employing CCD multichannel detection. Images collected with the LCTF Raman chemical imaging system provide essentially diffraction-limited resolution, as verified with USAF test targets and quantitative CTF analysis. Raman images of model systems clearly surpass all previous Raman imaging data.

The LCTF Raman system is robust, not prone to thermal drift, and defines the state-of-the art in Raman imaging technology. It promises to usher in a new era of practical Raman imaging, and has the potential to revolutionize Raman microscopy. However, the devices produced to date represent only the first generation of LCTF Raman development. Outstanding LCTF Raman issues are to improve throughput, to develop improved filters for the red/near-IR range appropriate for use with He-Ne, Kr^+ or laser diode sources, and to exploit confocal volume Raman imaging; these issues will be addressed in Phase II.

Commercial Potential

Compared to existing, non-imaging systems, the LCTF Raman system adds the powerful ability to image chemical species in heterogeneous samples, and to map out spatial distributions and features. This is a key benefit in working with real-world samples, which tend to be heterogeneous on the spatial scales of interest.

In many cases, the spectral bands of interest are known, and the measurement consists of identifying the presence and/or location of various species in a sample. Present practice is to use non-imaging systems such as a scanned microspot, which provide rich spectral data but limited (or inefficient) collection of spatial data. Use of a LCTF Raman system is the ideal way to produce the desired data set, which was not previously practical to obtain. One expects that a LCTF Raman system would also include a conventional fiber feed to a dispersive spectrometer, to provide standard spectral data along with the new spectral imaging capability.

We anticipate that a turnkey Raman chemical imaging system would be a new and powerful tool, used broadly by industrial and academic researchers. Beyond the research market, the emergence of industrial markets depends on the utility of this approach in specific applications, which must be determined through evaluations and case-by-case comparison against present methods.

I.   Statement of Phase I Objectives

    i)   To use existing computer models to generate a tunable Lyot filter
        design optimized for Raman spectroscopy;

    ii)   To study the integration of such a filter into a microscope
        imaging system, including optical path, data acquisition, and
        camera requirements;

    iii)   To construct a fully imaging prototype filter according to this
        design, with resolution of 10 cm^{-1} or better, tunable over a range
        of 2500 cm^{-1};

    iv)   To characterize this filter for its spectral properties, including
        peak transmission, bandwidth, out-of-band rejection, and tuning
        range;

    v)   To test this filter to obtain true two-dimensional Raman
        spectroscopy images of test samples;

    vi)   To prepare a Phase I report describing the results.

II.   Summary Description of Research, Results, and Commercial Potential

II.a Research and Results
    i)   Computer model of Lyot filter for Raman spectroscopy
The filter was designed with a computer model based on the Jones
calculus of polarization state. Measured values of retardance and
dispersion are used for liquid crystal and mineral crystal elements. We
chose x-cut $LiNbO_3$ retarders for the high-order Lyot stages, and quartz
for the lower-order stages. A total of 18 elements were employed, each
of which is continuously tunable by means of liquid crystal variable
retarder elements. Modeled passband and out-of-band rejection are shown
in Figure 1.

    ii)   Integration with microscope
The system was constructed around an Olympus BHSM-2 microscope, as shown
in Figure 2. Epi-illumination is provided by an Ar^+ laser (Coherent CR-
3) coupled through a fiber before impinging on a high-performance
dielectric notch bandpass filter (OCA Microplasma) to reject the silica
Raman bands developed within the fiber. The conditioned laser light
fills the back aperture of the microscope objective and excites the
sample. Raman scatter is collected by the same infinity-corrected
objective and presented to two holographic notch filters which remove
laser back-scatter. The Raman signal then passes through the LCTF and
is imaged onto a cooled CCD camera (Princeton Instruments TE-CCD-768-
k/2). Raman light is also coupled efficiently via a swing-away mirror
to a fiber-optic bundle, prior to passing through the LCTF. The Raman

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fiber-optic is coupled to a dispersive Raman spectrograph employing multichannel CCD detection and allows the system to perform as an efficient confocal Raman microprobe when this is desired.

iii) Filter construction

The LCTF was built using similar techniques to those developed for previous filters with broader passband (a few nm). The only significant issue was a difficulty in obtaining the $LiNbO_3$ retarders needed to achieve a narrow passband. These parts have a large aspect ratio (up to 100:1), and the surfaces must be parallel to 5" of arc. We worked with a vendor to identify a polishing process which produced these parts with high yield. Subsequent filter construction proceeded smoothly.

iv)   Filter characterization

We tested the LCTF for bandpass, transmission, out-of-band rejection, free spectral range, and tuning accuracy using a 0.5m spectrometer. All were in good agreement with model predictions. The bandpass is 7.6 cm^{-1} and is free of sidebands; transmission ranges from 6.7 to 16.3 percent; rejection is $>10^4:1$ for out-of-band light; the filter is tunable over a range of 4600 cm^{-1}, in milliseconds, with no moving parts. Repeatability is 0.38 cm^{-1} over this range.

v)   Raman imaging of test samples

Figure 3a shows the spectrum of a polystyrene microsphere, taken with the LCTF by tuning it sequentially to various wavelengths and recording the image intensity; Figure 3b shows a spectrum of the same samples taken with a non-imaging Raman microprobe and a dispersive spectrometer. Note the agreement of spectral features and relative intensities in the two Figures, indicating excellent spectral performance by the LCTF.

Figure 4 shows a brightfield image of the 2μ microspheres, taken with a 100x microscope objective, which demonstrates diffraction-limited spatial resolution. Airy rings are visible in this image, taken through the LCTF. A Raman image at 992 cm^{-1} is shown as Figure 5, with a 500 nm size bar shown for reference. The quality of this image is unmatched in Raman imaging to date.

II.b Commercial potential

Compared to existing, non-imaging systems, the Raman LCTF system adds the novel ability to visualize the distribution (morphology and architecture) of chemical species in heterogeneous samples with molecular compositional specificity. Raman images can be collected rapidly, non-invasively, with limited or no sample preparation, at high spatial resolution (< 250 nm) and with high fidelity where the number of image pixels is limited by the number of pixels on the CCD detector. Most important, every image pixel has a Raman spectrum associated with it whose quality is identical to that obtained with conventional non-imaging spectrometers. These key benefits make Raman imaging practical for the first time in the analysis of real-world samples, which are chemically heterogeneous on the spatial scales of interest.

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With the development of the LCTF Raman system, it is now practical to collect simultaneously high spatial/spectral resolution data sets of complex unknown materials having millions of pixels. The LCTF Raman imaging system is the ideal way to produce the desired data set, which was not previously practical to obtain. At the same time, the capability to perform conventional Raman microspectroscopy is intrinsically valuable, and one expects that a commercial Raman LCTF system would also include a fiber-optic feed to a dispersive spectrometer, to provide standard Raman spectral data along with the new spectral imaging capability.

We anticipate that such a turnkey Raman chemical be a new and powerful tool, used broadly by industrial and academic researchers for material analysis. In addition, we anticipate that process chemists would take full advantage of the Raman chemical imaging approach to perform at-line failure analysis and ultimately to perform non-invasive in-line Raman imaging of materials during the production process. The emergence of industrial markets, in particular the process monitoring markets, awaits beta-site research with industry partners to quantify benefits of the LCTF approach in specific applications.

III.   Detailed Description of Research and Results

i)   Design LCTF optimized for Raman imaging
The first step was to model a Lyot filter with the desired bandwidth and free spectral range. A Lyot stage consists of a retarder, liquid crystal tuning element, and a linear polarizer, while a Lyot filter[1] consists of several stages placed optically in series. The filter has a spectral resolution which is determined by the FWHM of the thickest retarder element, while unwanted adjacent orders are blocked by subsequent thinner stages. Thus the overall free spectral range (FSR) is set by the thinnest retarder element. Excepting for dispersion, the bandwidth of a retarder is constant in wavenumbers, or quadratic in λ, and a Lyot filter behaves in like manner.

In designing the Raman LCTF, existing models and techniques were used, which have been repeatedly verified in work with filters having broader bandpass. These use the Jones calculus[2], which tracks the polarization state for forward-propagating light. The model ignores reflections at interfaces, which has not been observed to introduce errors, as all components are index-matched in the final assembly. Dispersion values for the liquid crystals and fixed retarders are based on measured values, as are the wavelength-dependent polarizer transmission and extinction $k_1(\lambda)$ and $k_2(\lambda)$.

One new concern was the need for high-value retarders, to provide the high spectral resolution sought. Use of such components brings with it possible problems of field-of-view and thermal stability. A second concern was that, because the ratio of FSR : FWHM is high, a large number of stages would be required, leading to low transmission and overall complexity. These concerns are now discussed in detail.

$LiNbO_3$ was chosen for the high-value retarders, for the following reasons:
   a) it is readily available in apertures of 30 mm or more;

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b)   it has a high index of refraction $n_o \approx 2.30$;
c)   it exhibits moderate birefringence $\delta n \approx 0.09$; and,
d)   LiNbO$_3$ can be fabricated using conventional methods.

The first and last insure that one can obtain the material and have waveplates
built. The high index yields a wider field-of-view, since off-axis spectral
shifts scale as $1/n_o^2$. The third factor is important, because if the
birefringence is too low, overly thick elements are required and the filter
becomes unwieldy. For example, if the highest order stage were quartz, it
would have to be 3.5 cm thick, which is not practical. On the other hand,
high-δn materials bring other fabrication problems: to achieve uniformity
across the aperture with a high-δn material, the parallelism requirement
becomes extreme. In all these regards, LiNbO$_3$ appears to be a superior choice
to calcite except when constructing very narrowband (sub-Angstrom) filters.

Models of off-axis performance indicated that, while superior to other
materials, LiNbO$_3$ would still exhibit unacceptable off-axis spectral shifts.
For this reason, wide-field elements were used for the thickest five retarder
elements. These consist of a pair of elements, each with half the required
thickness, separated by a half-wave plate. The two elements are oriented with
their crystal axes crossed, and the half-wave plate is oriented at 45°. This
arrangement, often used in narrowband solar H$_\alpha$ filters, greatly increases the
field of view compared to a simple retarder element. Models indicated that
this would yield a +/- 7° field-of-view, well in excess of the requirement.

The problem of thermal drift is not so easily solved by design. Thermal drift
in wavelength, per C°, is a significant fraction of the FWHM of the filter.
Rather than devise a thermally stabilized enclosure, the design used a
thermistor in the filter housing to monitor the actual temperature of the
filter elements;   this is sensed by the control electronics module, which then
adjusts the drive voltage to the liquid crystal elements, and actively
compensates for any drift in the retarders.

To address the need for a large number of stages, two approaches were used.
First, the filter was constructed in two detachable modules, one containing
the highest-order elements, and one with the lower-order elements. This
simplified the construction, and avoided problems in handling a single
unwieldy component. The two modules may be joined to form a single, light-
tight assembly. Second, to minimize the losses inherent in a many-stage
filter, a high-transmission polarizer was used. Optical losses are below 2%
per liquid crystal element at these wavelengths, and the retarders are nearly
lossless, so polarizer absorption is the dominant term in determining overall
filter transmission. Since the Raman system was to be used over the range 500
- 650 nm, where polarizer materials exhibit high dichroism, a lightly dyed
material could be used, to provide higher transmission while still insuring
adequate extinction. Most common polarizer material, like Polaroid HN38, is
more heavily dyed to avoid leakage in the violet spectral range;   this cuts
transmission unnecessarily.

Based on this choice of materials, a design was developed for an 18 stage Lyot
filter. It meets the bandwidth, tuning range, stability, field-of-view and

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image quality requirements for Raman imaging. The filter constructed in Phase
I was built to this design, and performed as predicted.

The principal limitation of this design is its relatively low transmission,
which ranges from 7 to 16 percent. A novel design was devised to overcome
this problem, and a model was developed of this new design. Based on the
Evans split-element retarder, it should nearly treble the throughput of the
LCTF, as described in Section IV, Key Improvements for Phase II.

ii)   Integration into microscope
The first task in integrating the LCTF into a Raman imaging system was the
selection of a laser excitation source. Normally, each application brings
with it factors that determine the optimal excitation wavelength; however,
given the budget limitations of the Phase I effort, a single Ar^+ laser
wavelength of 514.5 nm was chosen due to its availability in Dr. Treado's
laboratory. Integration of the laser source into an existing upright optical
microscope was achieved by coupling the laser source via a 200 μm core quartz
fiber optic as the illumination source transfer line. Fiber-optic coupling
enables uniform illumination of the sample. The output of the fiber-optic is
collimated and then filtered using a high performance dielectric bandpass
filter to remove silica Raman bands that develop in the fiber-optic. The
filtered light is presented to the rear aperture of the infinity-corrected
microscope objective, which produces uniform Koehler epi-illumination (180°
backscattering) of the sample. In the epi-illumination configuration, the
sample is illuminated and the Raman signal is collected through the same
infinity-corrected objective. Two holographic filters are used to reject the
Rayleigh scatter from the sample and to transmit the Stokes (red-shifted)
Raman image through the LCTF and to optics that project a magnified image of
the sample onto the CCD detector. A swing-away mirror provides access to the
Raman light prior to the LCTF and redirects it to a relay lens which couples
it into a fiber bundle. The fiber bundle feeds it into a dispersive 0.5m
spectrograph with multichannel CCD detection, and allows the system to perform
as an efficient confocal Raman microprobe.

A principal requirement in Raman imaging is to detect the weak Raman signal
levels efficiently, in the presence of much brighter laser excitation and
interfering background (usually fluorescence) signals. The holographic notch
rejection filters provide rejection efficiency of at least $10^{12}:1$ for the
Raman vs. the Rayleigh scattering. In addition, the four orders of rejection
provided by the LCTF allows discrimination of weak Raman features in the same
image field of view as intensely fluorescent species. Discrimination is
feasible with a Lyot filter due to its high rejection efficiency, in contrast
with tunable etalons that exhibit lower efficiency (100:1) and evidence
fluorescence artifacts in the putative Raman images. If fluorescence
background exists at the same wavelength as the Raman bands of interest, the
LCTF will not distinguish between the two signals. Rather, the microscopist
must rely on the use of high dynamic range detectors (16 or 14 bits) to
simultaneously record both signals. Using spectral subtraction, ratioing, or
multivariate techniques the fluorescence can be further discriminated from the
Raman information. In certain cases, fluorescence is too strong to be

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differentiated from weak Raman features.   The best strategy in this case is to
employ a different laser wavelength to excite the sample outside the
fluorescence excitation band.

The fiber optic feed to the spectrometer is provided because the capability to
perform conventional Raman microspectroscopy is intrinsically valuable.   One
expects that a commercially viable Raman LCTF system would also include a
fiber-optic feed to a dispersive spectrometer, to provide standard Raman
spectral data along with the new spectral imaging capability.

iii) Filter construction
A diagram of the filter is provided in Figure 6.   The liquid crystal optics,
polarizers, and quartz retarders were built using techniques developed for
other, broad-passband LCTFs.   New techniques were developed to permit use of
$LiNbO_3$ retarders, as follows.

First, while obtaining suitable $LiNbO_3$ was straightforward, there were
difficulties having it polished with suitable flatness and parallelism.
Single-sided polishing methods gave good finish and flatness, but poor
parallelism, while double-sided polishing tended to chip the parts or to round
the edges.   These problems were most serious when fabricating the thinnest
retarders (0.013" thick), due to the high diameter:thickness ratio of 96:1.
Tests were made of different polishing compounds and machine settings, and a
process was identified which produced good parts, thus solving the problem.

Second, the high index of $LiNbO_3$ had to be accommodated.   Normal LCTF filters
are assembled using index-matching epoxy, to join the many elements, and to
eliminate reflections at boundaries.   This reduces the mechanical complexity
of the resulting system, and improves the optical performance.   This approach
is quite effective when all materials have similar indices;   however, $n_o =$
2.30 for $LiNbO_3$, so parts made from this material had to be antireflection
coated to match their impedance to that of the epoxy ($n \approx 1.51$).   While not a
common coating, it was available with a reflection < 0.5% over the range 500 -
750 nm.   To ensure there would be no adhesion problems or chemical
interactions, a test blank of $LiNbO_3$ was made, coated, and epoxied to
precision BK-7 windows.   No problems were observed in this test piece, or in
the prototype filter work.

Next, the wide-field retarder design required $\lambda/2$ waveplates.   These were
constructed using polyvinyl alcohol (PVA) sheet retarder, stripped of its
plastic lamination using the method of Title, and glued between borosilicate
windows.   The nominal retardance was 280 nm, so optimum performance was at 560
nm.   In this design, the effect of $\lambda/2$ waveplate retardance error is to reduce
the peak transmission, rather than to add leakage.   An improved method might
be to use achromatic $\lambda/2$ plates, using the design of Pancharatnam, which would
improve the peak transmission.   However, there is an even better method based
on the Evans split element approach, as discussed below.

Finally, all retarders (quartz and $LiNbO_3$) were characterized to identify the
spectral locations λ_m of their maxima, for which $R = m\lambda_m$.   These are used to

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define the wavelength scale of the filter. For the high-order retarders, this was performed on a 0.5M spectrometer, to provide sufficient resolution (0.02 nm). Assembly proceeded normally, and the filter was completed without incident.

iv)   Filter characterization
Figure 7 shows the filter bandpass when tuned to 532 nm. The bandwidth, defined as the full-width at half-maximum (FWHM) is 0.20 nm, or 7.6 cm^{-1}. Peak transmission is 11 percent, and average out-of-band leakage is 0.01% over the range 500 - 650 nm. All are in agreement with model predictions. Repeatability was assessed by cycling the LCTF from one wavelength setting to another, then back. Spectra were taken of the LCTF transmission, with a fixed-grating CCD spectrometer, to measure any variation in passband after cycling. Shift was 0.38 cm^{-1}, or 0.01 nm, which is within the measurement error of the test.

Image quality was assessed in three ways. First, a USAF 1951 resolution test target was imaged in bright-field illumination, with and without the LCTF present. These are presented as Figure 8a) and 8b). No image degradation is visible. The center-to-center spacing of the finest grid is 4.3μ. Second, 2μ polystyrene microspheres were imaged in bright-field illumination, as was shown earlier in Figure 4. Diffraction-limited performance was obtained, and Airy rings are plainly visible.

A more quantitative measure was obtained by determining the contrast transfer function CTF(ω), which assesses the contrast reduction when a square-wave intensity pattern of a certain spatial frequency ω is imaged through an optical element. To date, it has not been the practice when developing Raman microscopes to employ CTF analysis to assess imaging performance. This is due in large part to the ability of CTFs to reveal very subtle deficiencies in imaging systems, whereas previous Raman imaging approaches have exhibited resolution which is considerably worse than the diffraction-limit. It is anticipated that CTF analysis will, in time, become a standard method for assessing Raman imaging performance, just as it (and the related MTF) are in other imaging disciplines.

In general, the CTF for a series of elements is the product of the CTF of each optical element. The CTF was measured for the imaging system with no filter, with the LCTF, with an IR cutoff filter that blocks light with λ > 700 nm, and with a 532 nm narrowband dielectric filter. These were compared against the theoretical CTF for a diffraction-limited image. Data was taken using a 5x, 0.1NA objective and a 1951 USAF resolution target. The results were:

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Table 1.   CTF for various filters

filter type   contrast reduction
                  (relative to diffraction limit)

no filter   -28.2% +/- 13.6%
IR cutoff only   -12.2% +/- 10.6%
LCTF   - 6.7% +/- 6.3%
532 nm filter   - 2.3% +/- 3.5%

The greatest contrast reduction occurred when no filter was present, and is
due to residual chromatic aberrations in the microscope optics. Better
performance was obtained with the infrared rejection filter engaged, and
better still when a narrowband filter was used, because the resulting image
was taken in monochromatic light. The LCTF introduced slightly more contrast
reduction than the dielectric filter, but both performed quite well. In
conclusion, the image contrast degradation introduced by the LCTF, while
measurable with digital CTF methods, is minimal.

v)   Raman imaging of test samples
Raman images were taken of several samples including model systems comprised
of microspheres, and real-world samples including polymer thin films, polymer
blends, semiconductors, and corrosion thin films. Figure 9 shows a multi-
component corrosion sample, which includes KNO_3, TiO_2, and WO_3 films. A
bright-field image (A) reveals little information that would allow
discrimination of the components. By tuning the LCTF to the wavelengths
corresponding to the characteristic Raman bands of each individual species,
however, very specific information is obtained. Image (B) was obtained with
the LCTF tuned to the 1057 cm^{-1} stretch band of NO_3^-, and indicates regions of
KNO_3, while image (C) was taken at the 604 cm^{-1} band of TiO_2, and image (D)
shows the 797 cm^{-1} band of WO_3. Chemical specificity is obtained, and a high-
definition view revealing specific molecular components and their associated
morphology is produced. Each image is collected in 60 seconds.

Indeed, a complete spectral data cube was obtained, by taking successive
images of the corrosion sample, as the LCTF was tuned by 3 cm^{-1} wavelength
steps. The result is a near diffraction-limited image for which a complete
Raman spectrum is available at every point in the image. The molecular
composition of the individual components is evident more clearly in spectra
extracted from individual pixels in the data cube, plotted in Figure 10. The
availability of such detailed data is unprecedented in Raman chemical imaging.

Note that there is no smearing or image degradation evident in the corrosion
images. Further, the image registration is perfect despite tuning the LCTF.
This is a clear advantage of the LCTF over competing technologies such as
AOTFs, rotating dielectric filters, or tunable etalons. This implies that the
LCTF Raman microscope is suited to the use of numerical deconvolution confocal
Raman imaging employing widefield illumination, as discussed below in Section
IV. This numerical approach to volumetric imaging has been developed for

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fluorescence microscopy at discrete wavelengths, and requires a mechanically stable, high image-quality microscope.

One can quantify the amount of image shift which results from LCTF tuning. The only significant source of image shift is prism action; that is, wedge in the LCTF, coupled with dispersion in the materials employed, to produce a wavelength-dependent angular shift. Wedge in the retarder elements is of order 20 micro-radians, and dispersion in index is 0.015 over the spectral range involved. Thus, prism action in these components is utterly negligible: 0.32 microradians, or 0.06 " of arc. More important is wedge in the liquid crystal cells, polarizers, and other elements, as they are less tightly toleranced in manufacture. In all, the image shift in tuning is approximately 2" of arc, still well below the diffraction limit of any microscope objective.

IV.   Identification of Key Improvements
a)   filter transmission
In Phase I, we have constructed an LCTF designed for Raman use, and have demonstrated performance that is unmatched by competitive Raman technologies, which include AOTFs, tunable LC etalon, rotating dielectric filters, and confocal point-scanning Raman microprobes.

AOTFs were first studied for Raman microscopy by Dr. Treado several years ago and provide substantially degraded spatial and spectral performance relative to the LCTF. Tunable etalons provide low free spectral range, low transmission, and poor out-of-band rejection performance. Rotating dielectric filters exhibit low spectral resolution and introduce significant image shift during operation. At present, the only imaging technology that can arguably compete with the LCTF is the point scanning Raman microprobe, due primarily to the high spectral resolution obtainable with the point scan technique. The Raman microprobe employs a dispersive spectrograph in combination with a multichannel detector to rapidly collect Raman spectra from point-localized regions in the sample.

In a Raman microprobe, light from a point in the sample is dispersed across a CCD detector, and light of a large range of wavelengths is captured at once. Flux from spatial regions outside the point being imaged, is ignored. To acquire a complete spectrum takes only a single exposure of a CCD located at the exit focal plane of a spectrograph. The integration time to produce a given signal in counts per CCD element is determined by a number of parameters, including the laser power density at the sample, the concentration of the analyte, the scattering cross-section of the sample at the laser wavelength, the sample scattering volume, the collection efficiency of the microscope, the quantum efficiency of the detector, and the peak transmission of the spectrometer and its dispersion. The greater the dispersion, in nm/pixel, the longer an exposure is required. If an $m \times n$ image were desired, it requires stepping across the image, and taking mn total exposures. Roughly, the acquisition time scales linearly with dispersion (resolution), and quadratically with the spatial resolution sought (m or n).

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On the other hand, an LCTF-based imaging Raman system employs widefield
illumination, and views all regions of the sample simultaneously at a spatial
resolution limited by diffraction (~ 250 nm). During operation, the LCTF
transmits a single wavelength at a time, and absorbs the rest. To acquire a
complete $m \times n$ image takes only a single exposure, and the integration time to
produce a given signal level in counts per CCD element is determined by the
same sample, laser power density, and microscope parameters that were   .
considered above, except that the peak transmission of the LCTF and its
bandwidth replace the spectrograph transmission and its dispersion as
governing parameters. If the LCTF bandwidth is greater, a greater total flux
lands on the CCD, assuming the Raman bandwidth exceeds the LCTF bandwidth. If
a spectrum of k points were desired, a total of k exposures would be required.
For high spectral resolution, k is large and the LCTF bandwidth must be
reduced as well, so the acquisition time scales quadratically with resolution.

It appears that a non-imaging system is favored when:
- a complete (dense) spectrum is required rapidly; and,
- an image is not desired,
while an imaging LCTF system might be favored when:
- the species are known *a priori*, so only a reduced number of spectral
  points are sampled, to uniquely differentiate the analyte species; or,
- an image is desired.

Numerical considerations favor the LCTF by 50,000 : 1 when an image is
required and only a handful of spectral planes need be sampled. However, if a
dense spectrum is needed and imaging is not required, a microprobe will be
several hundred times faster at gathering purely spectral data. These
considerations are inherent to comparing dispersive vs. imaging filter
architectures, and the viability of the imaging approach as a tool for Raman
use will depend on the relative amount and type of information that a given
application requires.   .

Despite the inherent benefits of the LCTF for imaging, the peak transmittance
of the present device is less than optimal. Indeed, throughput is the primary
limitation of the present LCTF. While cooled CCD detectors have more than
adequate sensitivity to accommodate the reduced throughput of the LCTF for
many applications, long integration times (minutes) are required to collect
the Raman spectral image information. However, the peak transmittance can be
improved significantly in a second-generation device, and this will be a major
focus of the Phase II research effort.

There are two reasons for the low transmission of the Phase I prototype LCTF.
First, a Lyot filter operates in polarized light, and loses half of the input
signal at the first polarizer. Second, absorptive losses in the many optical
elements leads to low efficiency. These can both be addressed, as follows.

It is possible to use a polarizing beam-splitter (PBS) to separate the two
polarization components of incident light; to convert the unwanted component
by means of a $\lambda/2$ plate, so that it also passes through the LCTF; and, to re-
combine the components with a second $\lambda/2$ plate and a second PBS, after they
exit the LCTF. This arrangement has been built successfully for LCTFs used in

fluorescence microscopy. Losses in the PBS and other elements limit the
efficiency to 90% of the theoretical maximum. Still, this is nearly twice the
50% efficiency which is achieved when only one component is utilized. Image
quality suffers if the two beams are not well-registered upon re-combination,
but resolution of 1024 x 1024 pixels can be achieved readily without
degradation. Also, the use of PBS elements adds approximately 3 cm. of glass
to the optical path, but this can generally be accommodated in an infinity-
corrected microscope beam without trouble. This approach could provide a
factor of 1.8 in throughput over the present system.

Second, it is possible to use a design described by Evans[3] for use in solar H_α
and Ca_k filters to gain a further improvement of approximately 2:1. Recall
that the high-order stages are constructed using a so-called wide-field
construction, where two high-order retarders of equal thickness are placed
with their crystal axes orthogonal, on either side of a $\lambda/2$ plate, which is
oriented at 45°. This produces a wider field of view than a simple retarder.
However, it is possible to construct such a stage where the $\lambda/2$ plate is
replaced by one of the lower-order retarder elements, which is actively tuned
by a liquid crystal element so it exhibits n+1/2 waves of retardance at the
passband. This has several beneficial effects. First, it eliminates the need
for the $\lambda/2$ plate. Also, if the two high-order retarders R_{H1} and R_{H2} are well-
matched, the transmission of the stage is given by:

$$T(\lambda) = \sin^2(\pi(R_{H1} + R_{H2})/\lambda) \cdot \sin^2(\pi R_L/\lambda)$$

so it has the same effect as two simple Lyot stages, one with retardance R_{H1} +
R_{H2}, and one with retardance R_L. Such a stage, termed an Evans split element
stage, has the same spectral filtration as two simple Lyot stages. A single
stage was assembled to test this concept, and its actual transmission is shown
along with model data in Figure 11. The high-frequency period is developed by
the high-order retardance R_{H1} + R_{H2} (two 0.072" $LiNbO_3$ elements), while the
low-frequency period is due to R_L (0.060" quartz). Excellent agreement was
achieved, and this approach looks very promising.

Using this design, the number of stages is cut in half, with a corresponding
drop in the number of polarizers and in the filter's absorptive loss.
However, more retarder elements and liquid crystal elements are needed. While
these are optically efficient, the complexity is increased, as is the
stringency of optical tolerancing. These improvements, along with further
optimization of the materials used throughout the LCTF, are natural avenues
for improving the filter throughput. In the best case, it should be possible
to produce an LCTF with transmission of 30% or more, for unpolarized incident
light.

b)   longer wavelength range
Another area for improvement is the LCTF free spectral range, which is
presently limited to use with Ar^+ or doubled Nd:YAG laser sources. To operate
with 647 nm Kr^+ sources, or laser diodes, an LCTF would have to tune over the
range 650 - 1050 nm. These sources are used to reduce sample fluorescence,
especially in biomaterials, or when working with certain types of samples such

as semiconductors, to obtain greater source penetration depth. In addition, red laser sources take advantage of the higher quantum efficiency of CCDs to red radiation. Since the spectral resolution of an LCTF is constant in wavenumber as it is tuned, the present retarder design will give adequately high spectral resolution. The retarders and liquid crystal cells all exhibit high transmission in the near-IR, somewhat above their visible-range values.

The spectral restrictions in the present filter are due to the dichroic polarizer materials, the $LiNbO_3$ coatings, and the limited operating range of the $\lambda/2$ plates. The latter would be removed by use of the Evans split element design just described, and would then cover the necessary range. Dielectric coatings can be obtained to couple the $LiNbO_3$ to an n=1.51 index with R < 0.5% over this range. Thus, only the polarizer presents a significant problem to construction of LCTFs for use with these longer wavelength sources.

Conventional sheet polarizer material is unacceptably lossy for use in a multi-stage Lyot filter. We have worked with an outside vendor to develop a novel polarizer for the near-IR, with 1000:1 contrast and markedly improved transmission. This material is used in a commercial line of LCTF filters, and has proven to be reliable. Transmission of the IR materials is shown in Figure 12, alongside the very high-efficiency dichroic polarizer used in visible range LCTFs. Preliminary models indicate that a high-performance Raman filter built could be built using this material, with specifications for bandwidth, out-of-band blocking, and peak transmission that would be comparable to a visible-range device.

c)   confocal LCTF Raman imaging
Based on the high demonstrated image quality of the LCTF Raman system, we intend to explore its suitability for Raman confocal volumetric imaging, using a series of height-stepped exposures and numerical deconvolution techniques to develop a three-dimensional map of the sample. If successful, this would provide nondestructive depth profiling of chemical concentrations in samples, at a spatial resolution of ~250 nm, with minimal or no preparation. Such an instrument would be a powerful diagnostic tool for a great many industrial processes. It would be is especially valuable in depth profiling of semiconductor impurities and dopants, thin-film corrosion systems, adhesive bonding systems, and similar applications. This is a potential high-payoff area which could have a technical and commercial return vastly exceeding that of the normal (non-volumetric) imaging LCTF Raman.

V.   Assessment of Commercial Potential
There appears to be an emerging research market among spectroscopists, materials scientists, biomedical researchers, and process chemists for a commercial version of the Raman LCTF, and/or complete turnkey imaging Raman systems. Newly-available image information which the Raman LCTF makes available, has excited some 'visionary' users, who perceive great potential in this approach. Already, several such people have contacted us to arrange for sample tests or possible purchase of such equipment, citing specific research

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programs which would benefit from imaging Raman data. This encourages us to believe that the Raman LCTF has a solid future as a research instrument.

It remains to be seen whether the power of imaging Raman techniques will become significant in broader analytical chemical practice, or not. This depends as much on the nature of the applications involved, and what is gained by visualizing the structure and morphology of species, as it does on the performance of the LCTF *per se*. An imaging Raman system is a new tool, complementary to and different from a spectrometer, and its utility is not yet proven. To compete in the long-term, the imaging Raman approach must offer unique data which is perceived as critical to an application, or it must produce data similar to that of other instruments, but do so in a more efficient or convenient way.

Several applications have emerged where the imaging Raman approach appears to offer a compelling benefit of this type. One area is in silicon processing, to assess strain and lattice deformation, and others are in pharmaceuticals, paper products, steam power generation, and waste water treatment. A partial list of industrial partners, whose researchers have already contacted Dr. Treado to assess the LCTF Raman approach for use in their application, includes Kodak, Westinghouse, Lockheed Martin, Bayer Polymers, Bayer Consumer Products, ICI Materials, Unilever, Proctor & Gamble, Ford, PPG, Johnson & Johnson, Union Carbide, Medtronic, Alza, Eli Lilly, and Glaxo-Wellcome. If LCTF Raman were to establish itself in any of these areas, there would be significant markets (a few dozen instruments/yr) in industrial research and materials analysis as well as in pure research.

References

1. Lyot, B., Compt. Rend., <u>197</u>, 1593 (1933).

2. Jones, R. C., J. Opt. Soc. Am. 31, 488 (1941).
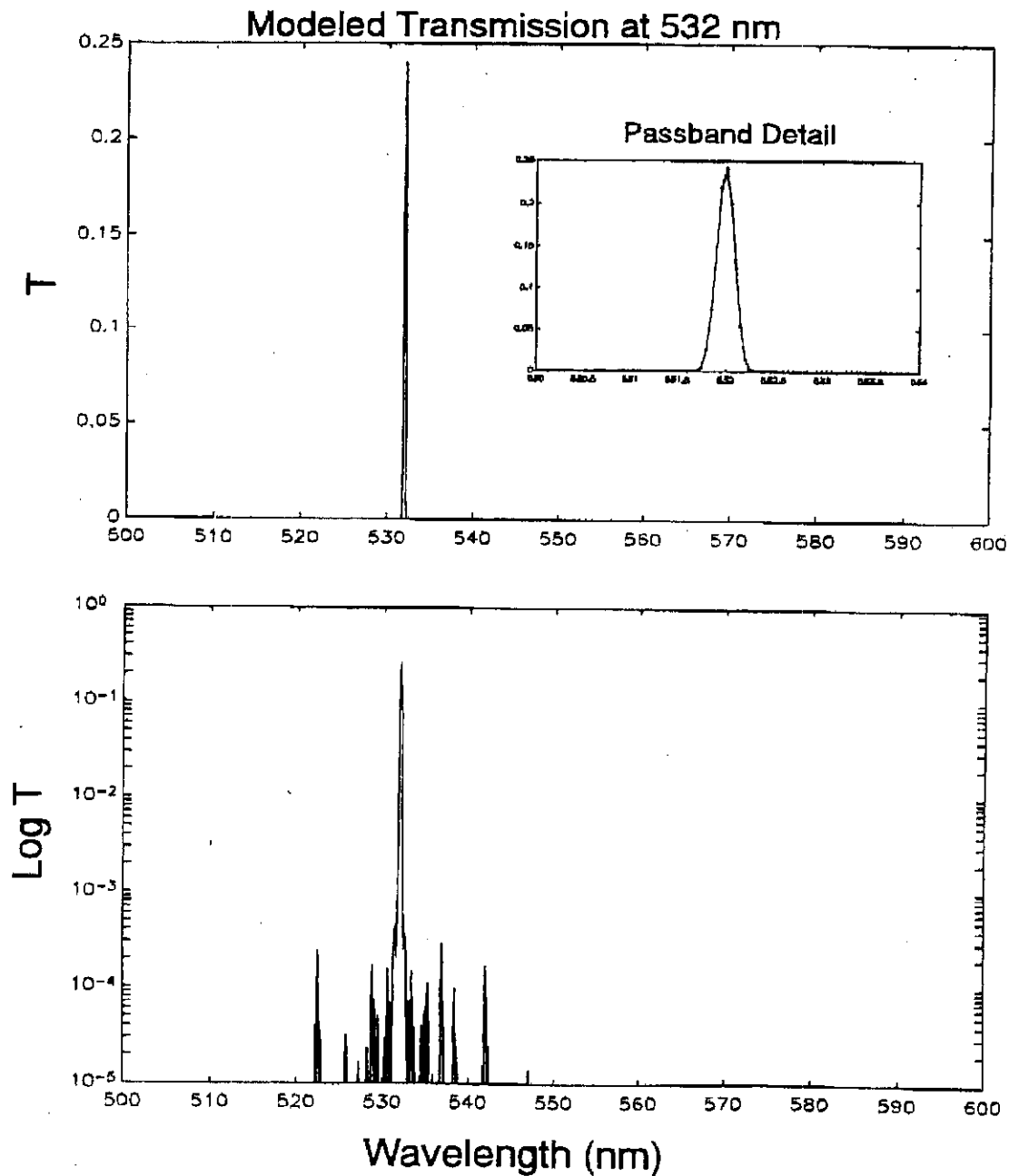
3. Evans, J. W., J. Opt. Soc. Am 48, 142 (1958).

Figure 1. Model LCTF performance when tuned to 532 nm, shown on linear and logarithmic scales. Passband detail is shown in the inset.
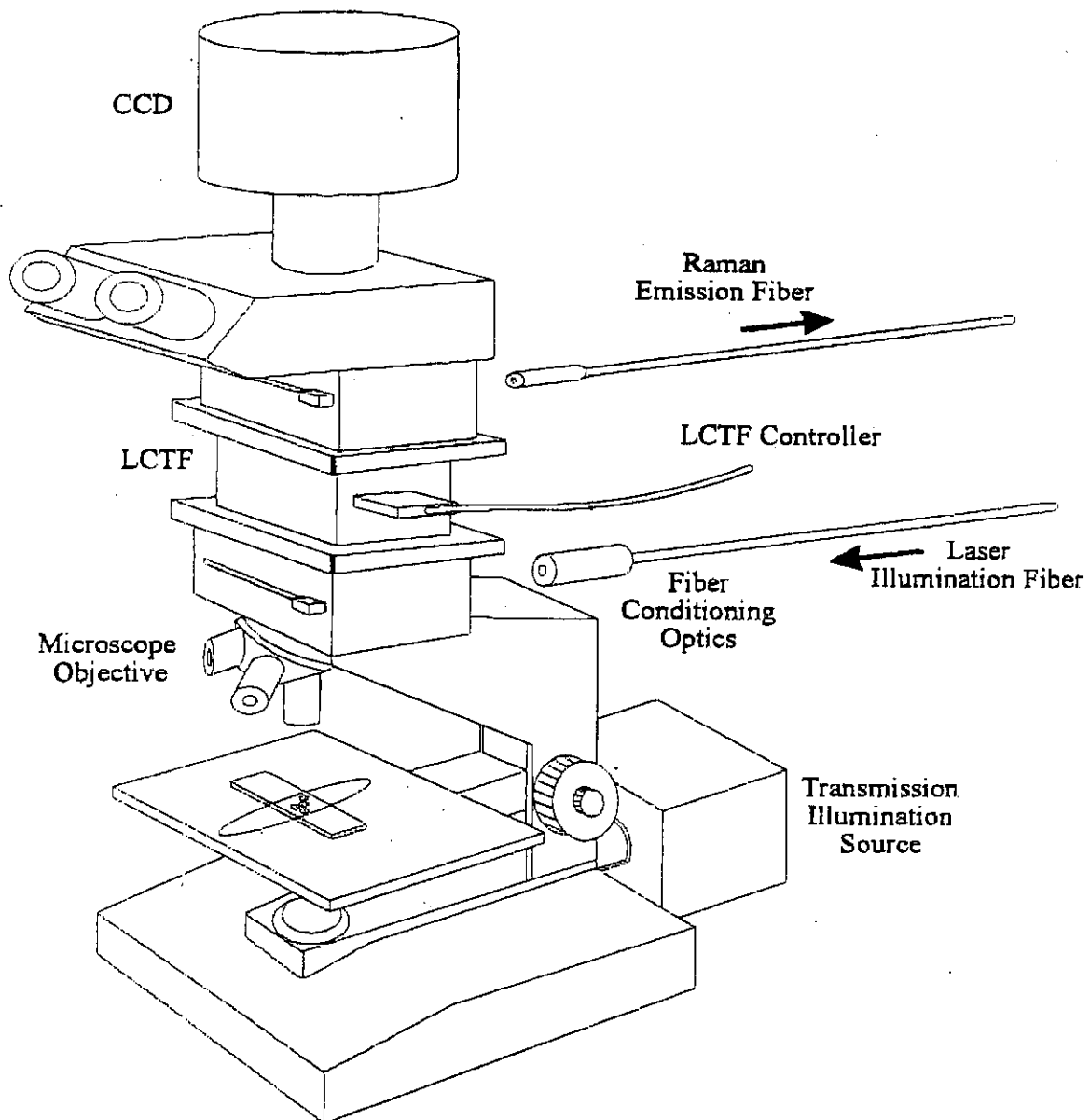
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CCD

Raman
Emission Fiber

LCTF Controller

LCTF

Laser
Illumination Fiber

Fiber
Conditioning
Optics

Microscope
Objective

Transmission
Illumination
Source

Figure 2. Diagram of the LCTF Raman filter integrated onto the
optical microscope.

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A

Wavenumbers (cm^{-1})

B

Wavenumbers (cm^{-1})

Figure 3. The top figure shows a Raman spectrum of polystyrene microsphere, obtained with the LCTF, while the lower figure shows the Raman spectrum of the same sample taken through a conventional (non-imaging) dispersive spectrometer system.
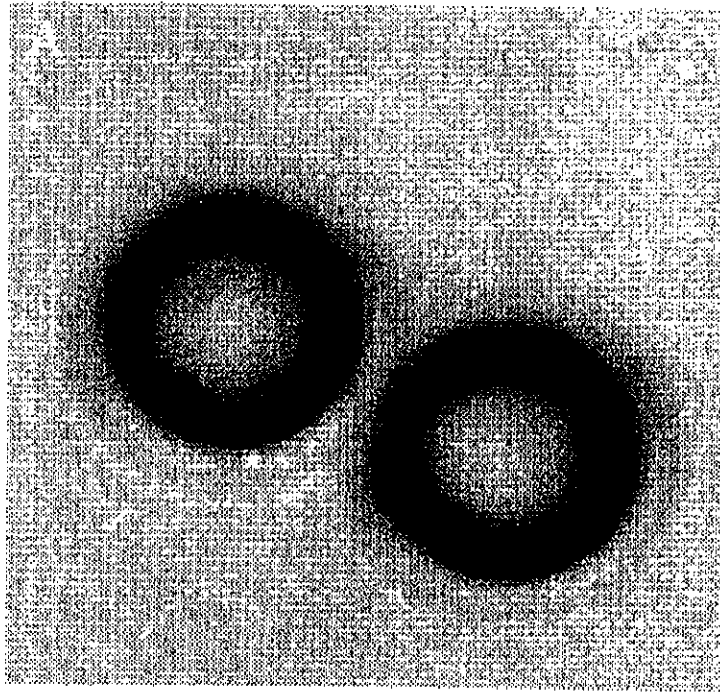
16

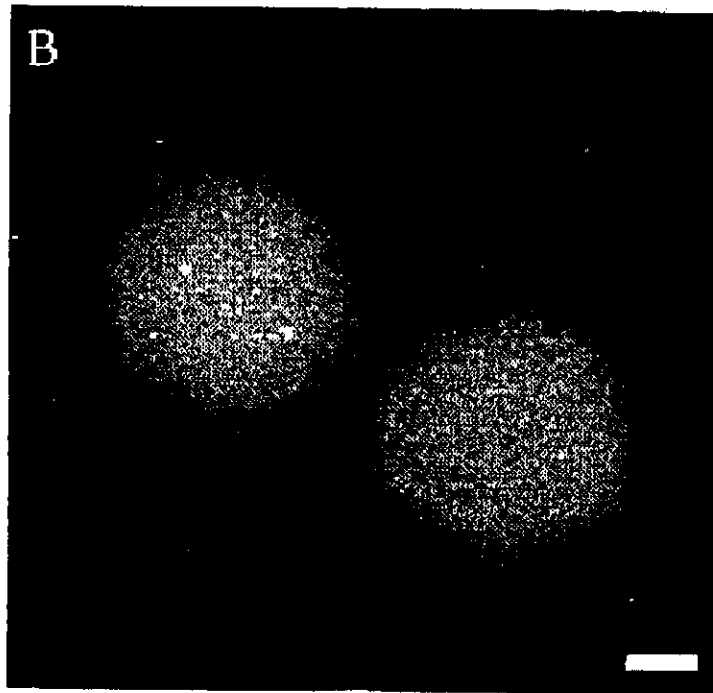Figure 4. Diffraction-limited image of 2 μm polystyrene spheres (brightfield).

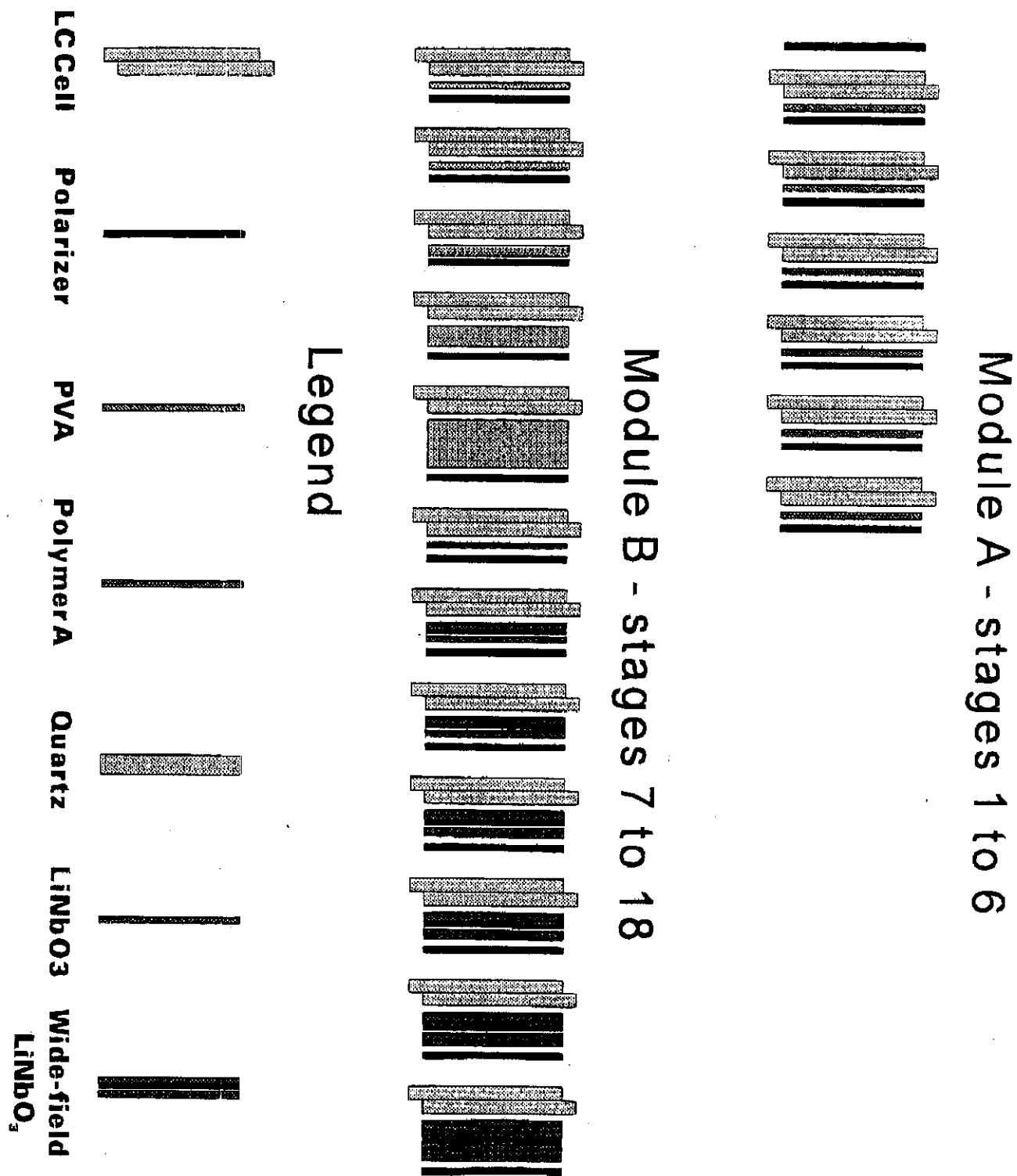Figure 5. Raman image at 992 cm-1 of the same sample. Note 500 nm size bar shown for reference.

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Figure 6. Diagram of the optical components of the Raman LCTF filter.
Thicknesses of components have been increased for clarity (not to scale).
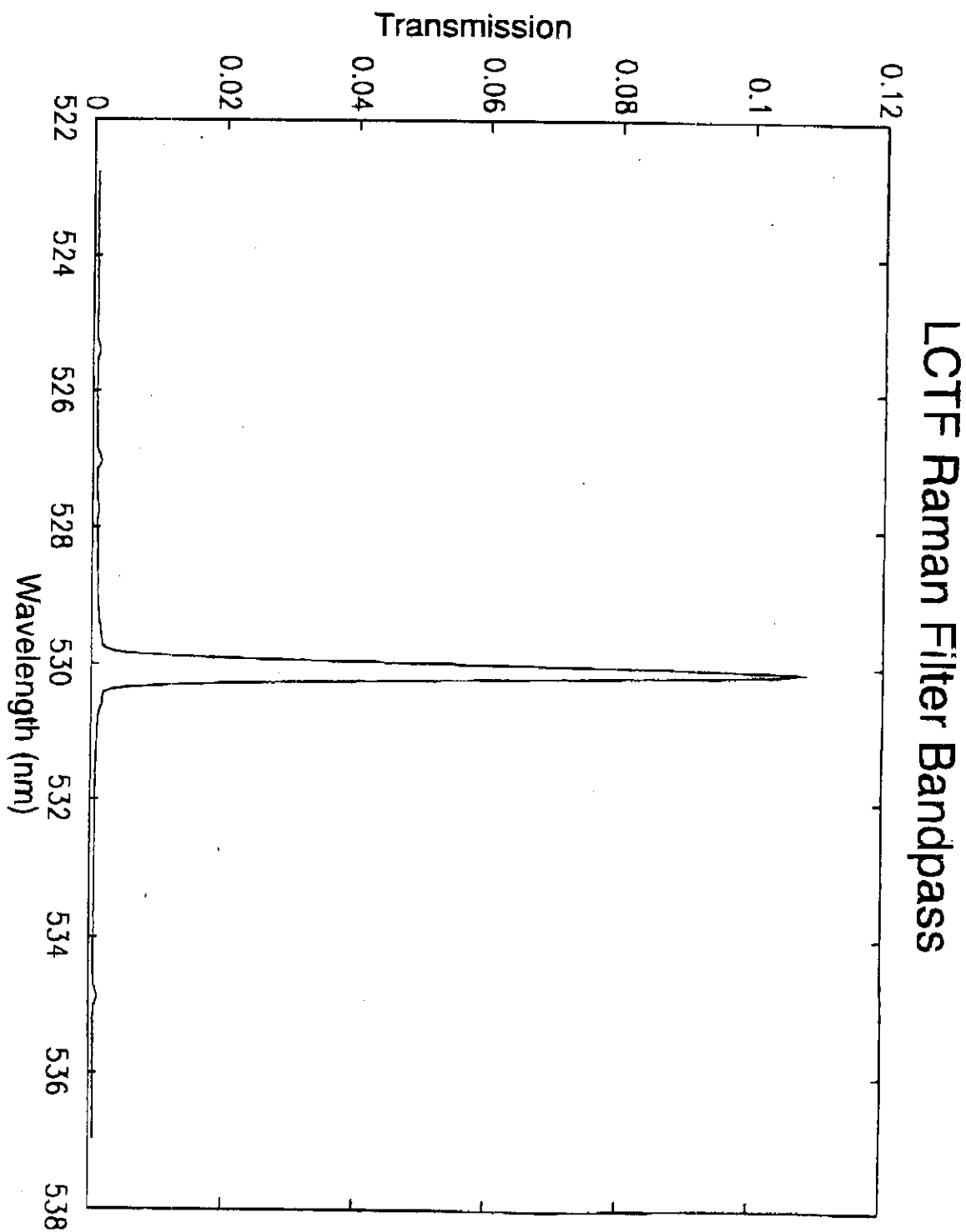
18

LCTF Raman Filter Bandpass

Figure 7. Passband of the prototype LCTF Raman filter, tuned to 532 nm. FWHM of the filter (corrected for the resolution of the test spectrometer) is 0.20 nm.
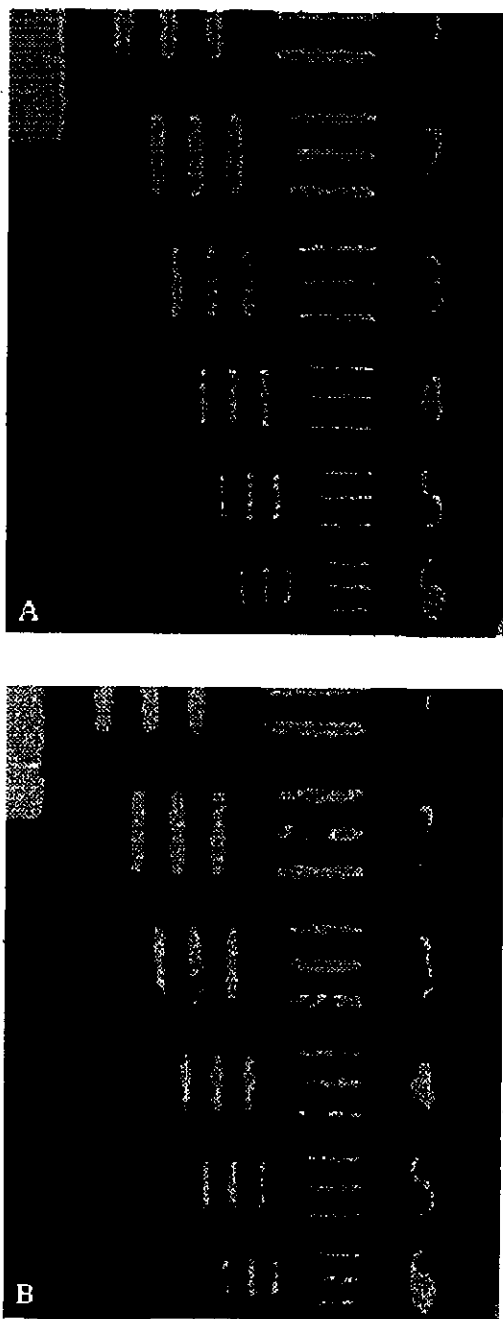
Figure 8. Images of a 1951 USAF resolution target without (top) and with (bottom) the LCTF Raman filter. Center spacing of finest grid is 4.3 μm. No degradation is apparent in the digital images.

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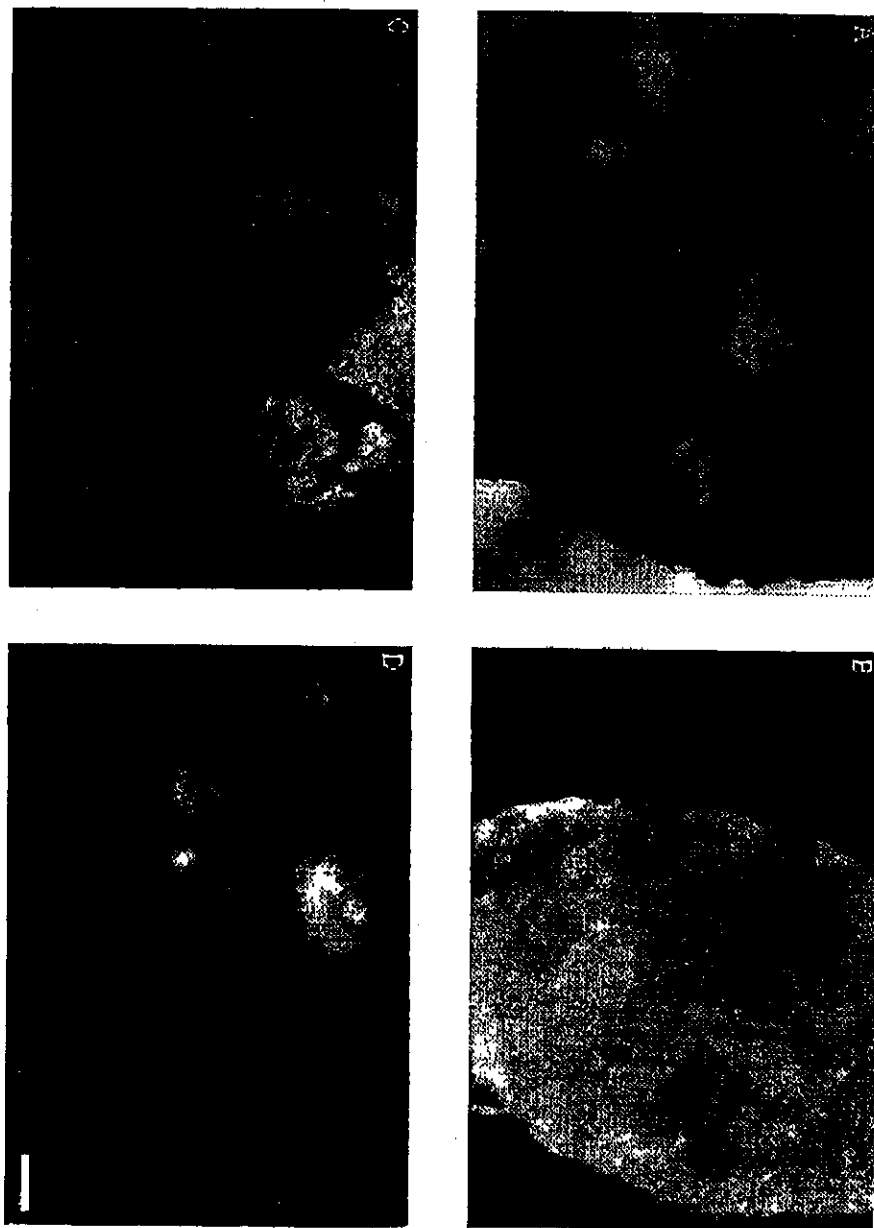Figure 9. Images of corrosion sample in (A) brightfield; (B) the 1057 cm^{-1} stretch band of NO$_3$; (C) the 604 cm^{-1} band of TiO$_2$; and, (D) the 797 cm^{-1} band of WO$_3$, showing chemical specificity as the filter selects each analyte in turn.

21
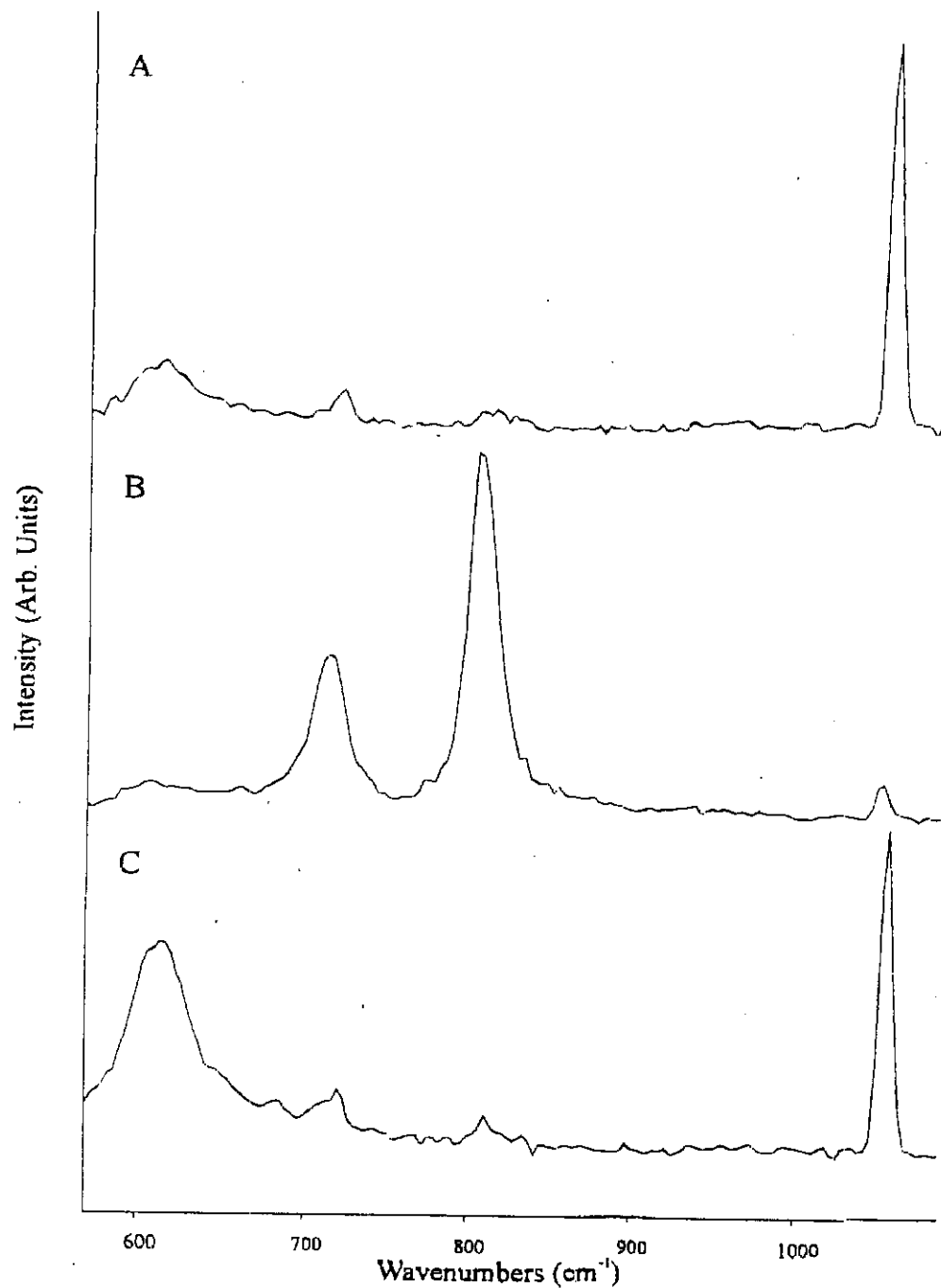
Figure 10. Spectra from individual pixels of Figure 9, showing (A) KNO3; (B) WO3; (C) TiO2. Full spectra exist for each point in the sample.

22
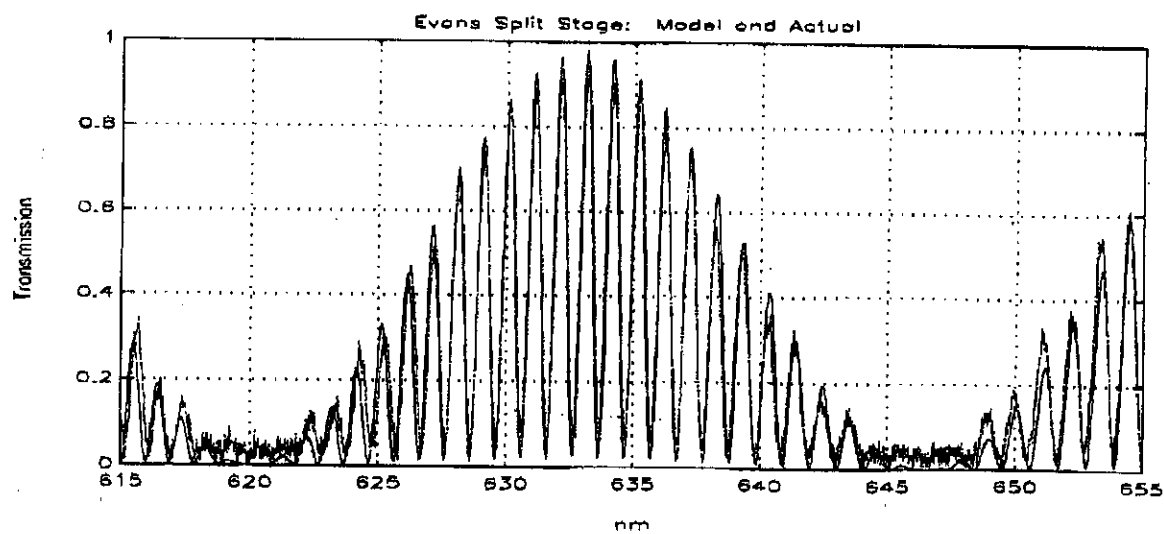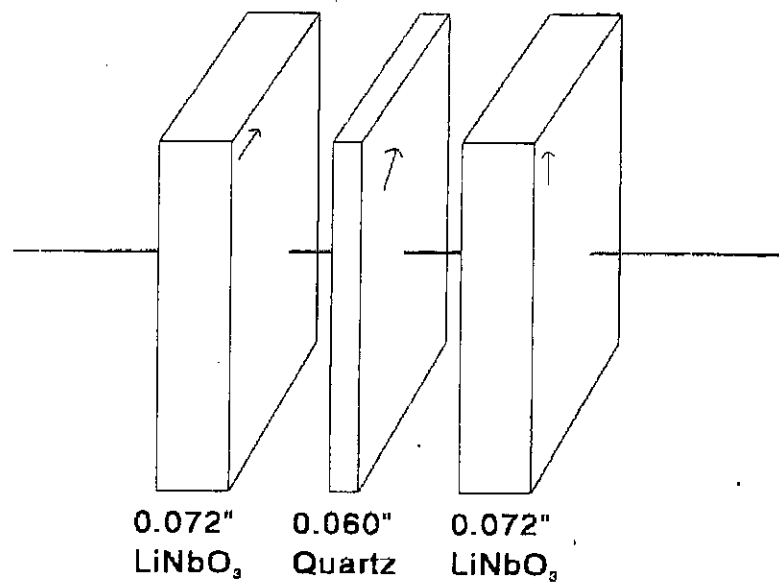
Figure 11. Above is pictured a diagram of an Evans stage, along with model
and actual data from a test piece. The high frequency spectral resolution is
due to the LiNbO3 while the low-frequency envelope is due to
to the quartz. It can replace two simple stages in a Lyot filter.

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Visible and Near-IR Polarizer Materials
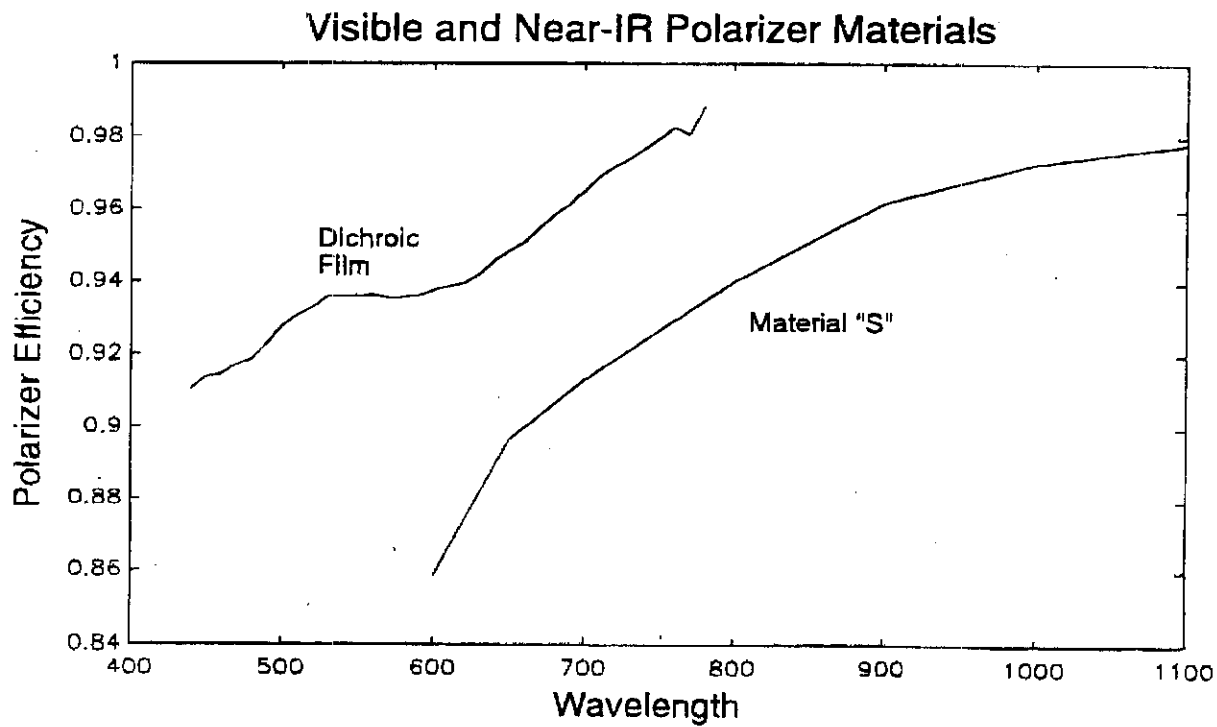
Figure 12. Transmission of single layers of visible and near-IR polarizer material proposed for use in second-generation LCTF red/IR devices. All transmission data are net of Fresnel losses.

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APPENDIX B

NATIONAL SCIENCE FOUNDATION
SBIR PHASE II PROPOSAL COVER PAGE
Small Business Innovation Research

TOPIC NO. 2	SUBTOPIC LETTER (If any) B	TOPIC TITLE Chemistry

PROPOSAL TITLE

High-Definition Raman Imaging Microscope

| NAME OF PROPOSING SMALL BUSINESS CONCERN Cambridge Research & Instrumentation Inc.	ADDRESS (Including ZIP CODE)
EMPLOYER IDENTIFICATION NUMBER (EIN) OR TAXPAYER IDENTIFICATION NUMBER (TIN) 04-2868535	21 Erie Street Cambridge, MA 02139

REQUESTED AMOUNT $ 300,000	PROPOSED DURATION 24 months	PERIOD OF PERFORMANCE 8/1/97 – 7/31/99

THE SMALL BUSINESS CONCERN CERTIFIES THAT:

	Y/N
1. It is a small business as defined in the SBIR Phase I - Phase II Instruction Guide.	Y
2. It qualifies as a socially and economically disadvantaged business as defined in SBIR Phase I - Phase II Instruction Guide. FOR STATISTICAL PURPOSES ONLY	N
3. It qualifies as a women-owned business as defined in SBIR Phase I - Phase II Instruction Guide. FOR STATISTICAL PURPOSES ONLY	N
4. NSF is the only Federal agency that has received this proposal (or an overlapping or equivalent proposal) from the small business concern. If No, you must disclose overlapping or equivalent proposals and awards as required by SBIR Phase I - Phase II Instruction Guide. (See Section Part III, Subsection D.1 (I))	Y
5. A minimum of one-half of the research will be performed by this firm in Phase II.	Y
6. The primary employment of the principal investigator will be with this firm at the time of award and during the conduct of the research.	Y
7. It will permit the government to disclose the title and technical abstract page, plus the name, address and telephone number of a corporate official if the proposal does not result in an award to parties who may be interested in contacting you further information or possible investment.	Y
8. It will comply with the provisions of the Civil Rights Act of 1964 (P.L. 88-352) and the regulations pursuant thereto.	Y

PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR

NAME Peter J. Miller	TITLE Staff Scientist	
SOCIAL SECURITY NO. 195-44-3236	TELEPHONE NO. (617) 491-2627	
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NAME Peter V. Foukal	TITLE President	TELEPHONE NO. (617) 491-2627

COMPANY OFFICER (FOR BUSINESS AND FINANCIAL MATTERS)

OTHER INFORMATION

PRESIDENT'S NAME Peter V. Foukal	YEAR FIRM FOUNDED 1985	NUMBER OF EMPLOYEES AVERAGE PREVIOUS 12 MO.: 16 CURRENTLY: 18

PROPRIETARY NOTICE See Part III, Subsection D.1 for instructions concerning proprietary information.
(Check here ☐ if proposal contains proprietary information.)

NOTE: The signed Certification Page must be included immediately following this Cover Page with the original copy of the proposal only.

Proposal Page No. 1

NSF FORM 1207 (SBIR) (6/96)

B-1

APPENDIX C

National Science Foundation
Small Business Innovation Research Program

PROJECT SUMMARY

NSF PROPOSAL NO.
DMI-9560600

NAME OF FIRM	
	Cambridge Research & Instrumentation, Inc.

ADDRESS	
	21 Erie Street
	Cambridge, MA 02139

PRINICPAL INVESTIGATOR (NAME AND TITLE)
Peter J. Miller, Staff Scientist

TITLE OF PROJECT
High-Definition Raman Imaging Microscope

TOPIC TITLE		TOPIC NUMBER AND SUBTOPIC LETTER
Chemistry		2.B

PROJECT SUMMARY

Project Summary

This Small Business Innovation Research Phase II project will build on the successful imaging Raman instrument demonstrated in Phase I, and produce second-generation instruments and software which fully exploit the potential of this new technique. In Phase I, chemical species were imaged using a narrowband liquid crystal tunable filter (LCTF) and CCD camera integrated into a Raman microscope. Diffraction-limited spatial images were obtained with complete (and spatially independent) Raman spectra at each pixel in the image, yielding an image cube with two spatial axes and one spectral axis. The novel LCTF approach renders high-definition Raman imaging feasible for the first time, and brings the power and specificity inherent in Raman spectral analysis to the imaging of chemical species.

In Phase II, key improvements will be made: transmission will be doubled (or more) based on a high-efficiency design identified in Phase I, and the long-wavelength limit will be extended from the present 700 nm to 1050 nm. Such an LCTF appears to offer near-revolutionary benefits in certain applications including semiconductor analysis, biomedical imaging, and pharmaceutical research. Performance in these areas will assessed experimentally in Phase II. Finally, data analysis methods will be developed to extract chemical species information from the large, spatially resolved datasets involved.

Commercial sales of LCTF Raman imaging systems are estimated at $21M over the next 5 years, for research, semiconductor, diamond, pharmaceutical, and power generation industries.

Key Words to Identify Research or Technology (8 maximum)
Raman, imaging, liquid crystal tunable filter

D.   Synopsis of Phase I Research Results

D.1   Overview
During Phase I, a liquid crystal tunable filter (LCTF) was designed, built,
and characterized for use in Raman chemical imaging. The filter has a design
bandwidth of 8 cm^{-1} and a tuning range of 500 - 650 nm, or 4600 cm^{-1}. Unlike
other tunable elements for Raman imaging, the LCTF is free of optical
distortions, spectral leakage, or image shift with tuning.

The actual filter performance met all model predictions, and was successfully
integrated into an optical microscope employing a 514.5 nm Ar^{++} laser source
coupled via fiber-optics to provide uniform illumination through infinity-
corrected microscope optics. Raman images were collected using a cooled CCD
camera. Sample spectra obtained through the LCTF are identical with spectra
of the same samples acquired with a conventional (non-imaging) dispersive
spectrometer employing CCD multichannel detection. Images collected with the
LCTF Raman chemical imaging system provide essentially diffraction-limited
resolution, as verified with USAF test targets and quantitative CTF analysis.
Raman images of model systems clearly surpass all previous Raman imaging data.

The LCTF Raman system is robust, not prone to thermal drift, and promises to
usher in a new era of practical Raman imaging. It is fair to state that LCTFs
define the state-of-the-art in Raman imaging technology and have the potential
to revolutionize Raman microscopy. However, the devices produced to date
represent only the first generation of LCTF Raman development. Outstanding
LCTF Raman issues are to improve throughput, and to develop improved filters
for the red/near-IR range appropriate for use with He-Ne, Kr^{++} or laser diode
sources; these issues will be addressed in Phase II.

Compared to existing, non-imaging systems, the LCTF Raman system adds the
powerful ability to image chemical species in heterogeneous samples, and to
map out spatial distributions and features. This is a key benefit in working
with real-world samples, which tend to be heterogeneous on the spatial scales
of interest.

D.2   Phase I objectives
The Objectives listed in the Phase I proposal were:

    i)   To use existing computer models to generate a tunable Lyot filter
            design optimized for Raman spectroscopy;

    ii)   To study the integration of such a filter into a microscope
            imaging system, including optical path, data acquisition, and
            camera requirements;

    iii)   To construct a fully imaging prototype filter according to this
            design, with resolution of 10 cm^{-1} or better, tunable over a range
            of 2500 cm^{-1};

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iv)   To characterize this filter for its spectral properties, including
      peak transmission, bandwidth, out-of-band rejection, and tuning
      range;

v)   To test this filter to obtain true two-dimensional Raman
      spectroscopy images of test samples;

vi)   To prepare a Phase I report describing the results.

D.3   Phase I results
The Phase I results are now presented for each of these objectives in turn.

i)   Computer model of Lyot filter for Raman spectroscopy
The filter was designed with a computer model based on the Jones
calculus of polarization state. Measured values of retardance and
dispersion are used for liquid crystal and mineral crystal elements. We
chose x-cut LiNbO$_3$ retarders for the high-order Lyot stages, and quartz
for the lower-order stages. A total of 18 elements were employed, each
of which is continuously tunable by means of liquid crystal variable
retarder elements. Modeled passband and out-of-band rejection are shown
in Figure 1.

ii)   Integration with microscope
The system was constructed around an Olympus BHSM-2 microscope, as shown
in Figure 2. Epi-illumination is provided by an Ar^{++} laser (Coherent
CR-3) coupled through a fiber before impinging on a high-performance
dielectric notch bandpass filter (OCA Microplasma) to reject the silica
Raman bands developed within the fiber. The conditioned laser light
fills the back aperture of the microscope objective and excites the
sample. Raman scatter is collected by the same infinity-corrected
objective and presented to two holographic notch filters which remove
laser back-scatter. The Raman signal then passes through the LCTF and
is imaged onto a cooled CCD camera (Princeton Instruments TE-CCD-768-
k/2). Raman light is also coupled efficiently via a swing-away mirror
to a fiber-optic bundle, prior to passing through the LCTF. The Raman
fiber-optic is coupled to a dispersive Raman spectrograph employing
multichannel CCD detection and allows the system to perform as an
efficient confocal Raman microprobe.

iii) Filter construction
The LCTF was built using similar techniques to those developed for
narrowband fluorescence filters. The only significant issue was a
difficulty in obtaining the LiNbO$_3$ retarders needed to achieve a narrow
passband. These parts have a large aspect ratio (up to 100:1), and the
surfaces must be parallel to 5" of arc. We worked with a vendor to
identify a double-sided polishing process which produced these parts
with high yield. Subsequent filter construction proceeded smoothly.

iv)   Filter characterization
We tested the LCTF for bandpass, transmission, out-of-band rejection,
free spectral range, and tuning accuracy using a 0.5m spectrometer. All

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were in good agreement with model predictions. The bandpass is 7.6 cm^{-1}
and is free of sidebands; transmission ranges from 6.7 to 16.3 percent;
rejection is >10^4:1 for out-of-band light; the filter is tunable over a
range of 4600 cm^{-1}, in milliseconds, with no moving parts.
Repeatability is 0.38 cm^{-1} over this range.

v)   Raman imaging of test samples
Figure 3a shows the spectrum of a polystyrene microsphere, taken with
the LCTF by tuning it sequentially to various wavelengths and recording
the image intensity; Figure 3b shows a spectrum of the same samples
taken with a Raman microprobe and a dispersive spectrometer. Note the
agreement of spectral features and relative intensities in the two
Figures, indicating excellent spectral performance by the LCTF.

Figure 4 shows a brightfield image of the 2μ microspheres, taken with a
100x microscope objective, which demonstrates diffraction-limited
spatial resolution. Airy rings are visible in this image, taken through
the LCTF. A Raman image at 992 cm^{-1} is shown as Figure 5, with a 500 nm
size bar shown for reference. The quality of this image is unmatched in
Raman imaging.

D.4   Identification and Significance of the Problem or Opportunity
Compared to existing, non-imaging systems, the Raman LCTF system adds the
powerful ability to visualize the distribution (morphology and architecture)
of chemical species in heterogeneous samples with molecular compositional
specificity. Raman images can be collected rapidly, non-invasively, with
limited or no sample preparation, at high spatial resolution (< 250 nm) and
with high fidelity where the number of image pixels is limited by the number
of pixels on the CCD detector. Most importantly, every image pixel has
associated with it a Raman spectrum whose quality is identical to that
obtained with conventional non-imaging spectrometers. These key benefits make
Raman imaging practical for the analysis of real-world samples, which are
chemically heterogeneous on the spatial scales of interest.

For many samples, unique, well-resolved Raman spectral bands of interest are
often known, or are readily identified by Raman microspectroscopy. The LCTF
Raman chemical imaging measurement then consists of identifying the presence
and/or location of analyte species in a sample by imaging at the
characteristic analyte Raman spectral bands. In general, it is not necessary
to have a complete Raman spectrum at each image pixel in order to characterize
sample chemistry and morphology. This is due in part to the intrinsic
redundancy of a typical Raman spectrum. Several regions of the spectrum can
be used to generate analyte-specific image contrast. For well-characterized
samples that require a limited number of spectral channels for analysis of
chemical content, but exhibit complex sample morphology, the LCTF approach is
clearly superior to competitive techniques because of the inherent efficiency
of analyzing all spatial channels simultaneously in a massively parallel
fashion.

Present practice is to use non-imaging systems such as a scanned microspot,
which yield complete spectral data but limited (or inefficient) collection of

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spatial data. Use of a Raman LCTF system is ideal for these cases, and yields
the desired data set, which was not previously practical to obtain. Since the
hardware required is similar to that used in standard microspot work, one
expects that a Raman LCTF system would also include a conventional fiber feed
to a dispersive spectrometer, to provide standard spectral data along with the
new spectral imaging capability.

For complex samples that are not well-characterized, past practice was to
collect high resolution Raman spectra at a limited number of sampling points
(pixels) using laser point scanning or line scanning Raman microprobes.
Again, these microprobes provided rich spectral data but inefficient
collection of spatial data. For example. point scanning would involve
focusing the laser to a small spot (~1 μm diameter) on a sample and rastering
it in the X and Y dimensions to construct a Raman image. If it required 1
second to acquire each point spectrum, it would take over 3 days to map a 512
x 512 pixel image. Of course, each image pixel would contain a complete Raman
spectrum.

Because the requisite sample acquisition time is impractical, point scanning
Raman imaging has not flourished despite having been commercially available
from a variety of instrument manufacturers since the mid 1970's. With the
development of the LCTF Raman system, it is now practical to collect
simultaneously high spatial/spectral resolution data sets of complex unknown
materials having millions of pixels. The LCTF Raman imaging system is the
only way to produce such a data set at present.

E.   Phase II Research Objectives

    The Phase II Objectives are the following:

    i)   to utilize improved designs to improve the transmission of Raman
          LCTF filters, resulting in a value at least twice that of the Phase
          I prototype;

    ii)   to develop a near-IR (NIR) version of the Raman LCTF which extends
          the long-wavelength operating limit from the present value of 700
          nm to a minimum of 1050 nm;

    iii) to construct and characterize a minimum of one visible-range and
          one NIR-range Raman LCTF which incorporate these improvements;

    iv)   to assess experimentally the benefits of Raman LCTF imaging in key
          areas including semiconductor, biomedical, and pharmaceutical
          measurements;

    v)   to make at least one of these LCTF instruments available to beta-
          site researchers for high-definition Raman imaging, on a revolving
          basis;

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vi) to develop data analysis methods which exploit the newly-available
     imaging spectroscopic information, and which render the large data
     sets tractable;

F.   Phase II Research Plan

The Research plan follows directly from the Objectives above, and is discussed
for each objective in turn.

i)   to utilize improved designs to improve the transmission of Raman LCTF
      filters, resulting in a value at least twice that of the Phase I
      prototype;

The two main sources of optical loss in the Phase I prototype are absorption
in the polarizers and imperfect waveplate action arising from the use of
simple $\lambda/2$ plates to construct the wide-field retarder stages. The former
term may be nearly halved, and the latter eliminated completely, by revising
the filter high-order filter stages from Lyot's first-type wide-field design
to the Evans split-element design[1] as described below. This will yield an
improved filter transmission ranging from 1.55 - 3.1 times that of the Phase I
prototype, depending on wavelength. It is believed that such a filter, when
built, will be the first tunable filter ever constructed using the Evans
design.

The prototype is a tunable Lyot design, consisting of an 18-stage filter where
each stage comprises a fixed quartz or $LiNbO_3$ fixed retarder; a tunable
liquid crystal waveplate; and a linear polarizer. In all, the light passes
through 19 polarizer elements, each of which has a transmission of ≈ 0.94, and
18 liquid crystal cells, each with a transmission of 0.98. So, cell and
polarizer losses set an upper limit to the filter transmission of

$$T_{upper\ limit} = (0.94^{19}) \times (0.98^{18}) \approx 0.20, \qquad\qquad \text{[Eq. 1]}$$

or 20 percent. Actual peak transmission was observed to be 16 percent at 600
nm, indicating some additional loss due to factors such as tuning error in the
liquid crystal elements and absorption in the retarders and epoxies.

Transmission at other wavelengths was lower, even after allowing for the
wavelength-dependent nature of the polarizer and cell losses. This is because
simple (non-achromatic) $\lambda/2$ waveplates were used in the wide-field $LiNbO_3$
stages. There were five such waveplates, made of polycarbonate film and tuned
for best operation at 610 nm. Over the LCTF tuning range 500 - 700 nm, the
efficiency of polarization conversion for these five elements in series ranges
from 0.4 - 1.0. Combined with the polarizer and cell losses, this results in
an overall transmission ranging from 8 - 20 percent, while actual values for
the Phase I prototype ranged from approximately 7 to 16 percent, mirroring the
range anticipated for this mechanism.

The 'split-element' design of Evans cuts the number of polarizers in half,
plus one, reducing this loss term significantly. Evans stages are used

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instead of the Lyot wide-field retarder stages, eliminating the λ/2 waveplates and their associated losses as well. The Evans design is illustrated in Figure 6: a high-order fixed retarder of thickness D and retardance R_h is constructed as two elements of thickness D/2, with their fast and slow axes crossed, and a low-order retarder of retardance R_1 is interposed with its fast axis at 45° to that of the D/2 elements. Each retarder has an associated liquid crystal tuning element. Such an assembly, placed between suitably oriented crossed polarizers, has a transmission of

$$T(\lambda) = \sin^2(\pi R_1 / \lambda) \cdot \sin^2(\pi R_h / \lambda), \qquad \text{[Eq. 2]}$$

for ideal polarizers with neither loss or leakage.

Again for ideal polarizers, the transmission of a single Lyot stage with retardance R between crossed polarizers is:

$$T(\lambda) = \sin^2(\pi R / \lambda), \qquad \text{[Eq. 3]}$$

so an Evans split-element stage is equivalent in its spectral structure to two Lyot stages. By constructing the LCTF as groups of Evans split stages, one collapses an N-stage Lyot filter into N/2 Evans stages, and reduces the number of polarizers by N/2.

Note that the number of liquid crystal tuning elements increases, since both of the D/2 half-elements in an Evans stage must be actively tuned, as well as the R_1 element, for a total of three tuning elements per Evans stage. The increase in liquid crystal elements is equal to the decrease in polarizers. However, the loss per polarizer is much higher than that of a liquid crystal cell, so the result is an increase in efficiency.

The Evans design offers a 55% improvement in throughput relative to the Phase I prototype, based purely on absorptive losses, and an additional improvement of 100% or more by eliminating the wide-field retarders and the attendant λ/2 waveplate chromatic error. In all, a transmission of 24.8% is anticipated, compared with the 6.7% - 16% of the Phase I prototype.

A single Evans stage was built during Phase I to confirm feasibility of construction, and it performed to predictions as shown in Figure 7. Such a design requires tighter tolerancing of the liquid crystal elements, compared to a Lyot design: the permissible error in the liquid crystal elements which tune the D/2 retarders are halved, as errors in either element contribute to wavelength error for the stage. Also, the extinction of the stage is limited to a level given by the expression

$$T_{min} = \sin^2(\pi(\delta R)/\lambda) \qquad \text{[Eq. 4]}$$

for the minima corresponding to the term $\sin^2(\pi R_1/\lambda)$ in Equation [2]. This is true even for ideal polarizers: the R_1 minimum occurs because the retardance of the two D/2 elements subtracts and cancels to produce a minimum; if they differ in retardance by δR, the cancellation is imperfect and the depth of the minimum is reduced.

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The liquid crystal retarders constructed for this work are readily tuned with an accuracy of +/- 6 nm anywhere within their operating range. This results in a worst-case δR of 12 nm, for an acceptably low T_{min} of 0.7% for the stage. Recall that the filter achieves a high rejection of out-of-band light not through extreme contrast in any single stage, but through the concerted action of several stages with low-to-moderate contrast. Models which use the Jones calculus rather than the simplified equations [2] and [3] confirm that overall filter leakage will not be unacceptably degraded through use of the Evans design.

Similarly, while better uniformity across the aperture on the fixed D/2 retarders is desirable, present materials appear adequate for use in an Evans design. The Phase I parts have +/- 9 nm variation across their aperture; the majority of this is radial due to spherical figure error in the pieces. This has only a slight impact on passband accuracy, and causes a center-to-edge shift in passband center of 0.24 cm^{-1}. Considering leakage, one can see that figure errors which are common to both D/2 parts, such as matched spherical power, will cancel when the retardance of the two D/2 elements subtracts (as occurs at the R_1 minima). So, improving the figure of these components will increase passband uniformity across the aperture somewhat, but will not have a significant effect on leakage. To the extent it is achieved, this improvement will occur through using the expanded schedule of the Phase II effort. During Phase I, there was insufficient time to iterate with the vendor and reduce the figure error in what were already quite acceptable pieces.

ii)   to develop a near-IR (NIR) version of the Raman LCTF which extends the long-wavelength operating limit from the present value of 700 nm to a minimum of 1050 nm;

The P.I. has developed a high-efficiency NIR polarizer during a previous NASA project directed towards remote sensing projects. It is a special version of a commercially-available polarizer, optimized for the NIR range extending to the end of silicon CCD response at 1050 nm. While conventional sheet-polarizer is heavily dyed and has relatively low transmission as a result, the transmission of this material is greater than 90% at all NIR wavelengths, and above 94% over the range 800 - 1050 nm. It is thus ideal for use with laser diode sources at 780 nm and longer. Data for this material and for a standard material are given in Figure 7.

Design of a high-performance NIR Raman LCTF will be based on the Evans design described above, to minimize the number of polarizers and their absorptive loss. In this spectral range, the liquid crystal tuning elements exhibit extremely high transmission (99% at 850), so the requirement of an Evans design for a larger number of these parts does not reduce LCTF performance greatly. Acceptable tuning error, retarder error, and optical figure error all scale directly with the wavelength, and so are more easily met in the NIR than the visible; at the same time, the bandwidth of an LCTF is constant (in cm^{-1}) with wavelength. That is, except for dispersion, the bandwidth of an NIR filter will be the same as an identical filter constructed for use in the visible. Accordingly, a design with resolution of approximately 8 wavenumbers

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is contemplated, using the same LiNbO$_3$ retarder components and dimensions as in the Phase I prototype.

iii) to construct and characterize a minimum of one visible-range and one NIR-range Raman LCTF which incorporate these improvements;

Techniques have been developed at CRI over the past eight years for the manufacture of the liquid crystal cells required for this work. Similarly, the assembly of these components together with polarizers, retarders, and precision windows into completed LCTFs has become highly advanced through the commercial sale of these instruments. With the introduction of a 'ruggedized' construction last year, these filters can survive temperatures from -20OC to +60C, and humidity of up to 80%. The electronics and techniques for tuning the liquid crystal retarder elements, based on an active capacitive sensing method, are also well-established. Software to calculate the required amount of liquid crystal tuning action in each stage, accounting for dispersion and thermal drift in the retarder elements, has been run successfully for four years. These areas are not seen as involving risk or needing further development during the Phase II effort, either for the visible or the NIR filter.

The two unusual requirements of Raman LCTF construction are to obtain high-order LiNbO$_3$ retarders, and to make a large number of spectral measurements at extremely high resolution. Based on the Phase I work, obtaining the retarders is practical although we will work to improve the figure slightly. Turning to the need for spectrometry, this is felt first during construction, when the quartz and LiNbO$_3$ retarders are scanned to provide a spectral calibration scale, but it also occurs when a filter has been completed, in order to measure its performance. At present, this is done using a Spex 0.5M scanning spectrometer with a chopper, lock-in amplifier and computerized data acquisition. This takes a great deal of time: a single spectral scan contains 1000 - 1500 points and requires a 1s integration time per point to get sufficient signal:noise ratio. Aside from the inconvenience, it is difficult to maintain retarders at a steady temperature in air for the required duration (20 minutes). Characterizing the leakage of an LCTF as it is scanned across its range requires one hundred such scans, or 33 hours of continuous scanning. Many important measurements are simply impractical as a result.

In Phase II, the scanning spectrometer will be replaced with a high resolution transmissive holographic instrument that covers the entire spectral range (500 - 750 nm for the visible filter, or 650 - 1050 nm for the NIR filter) across the active area of a 1" CCD detector. A resolution of 10 cm^{-1} per pixel is quite sufficient for the characterization of the LiNbO$_3$ retarders and other components, as these items have periodic, sinusoidal spectra; determination of the peaks can be fitted to considerably less than one pixel. Only one measurement, that of detailed bandpass shape, requires higher resolution. Note, however, that it requires only a small spectral range to be scanned, so it is quite practical to perform this on the scanning, rather than CCD-based, instrument.

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A CCD-based instrument will provide a spectrum in approximately 2-3 seconds, making it possible to characterize the LCTF much more completely. Also, debugging any problem in the filters will be enormously easier with such an instrument. Characterization will include measurements of peak transmission, bandpass shape, leakage of out-of-band light, free spectral range, and tuning accuracy, as in Phase I. Additional work in Phase II will focus on the response for off-axis rays and thermal drift measurements. Improved values for the thermal drift of $\delta n(\lambda)$ for $LiNbO_3$ and quartz will be sought and incorporated into the LCTF thermal drift compensation routines.

iv)   to assess experimentally the benefits of Raman LCTF imaging in key areas including semiconductor, biomedical, and pharmaceutical measurements;

Applications for a Raman imaging microscope are diverse because almost every manufactured or natural material has a unique, intrinsic Raman spectral fingerprint which can be harnessed to generate molecule-specific image contrast without performing invasive sample staining procedures. The broad applicability of Raman imaging is one of its many attractive features.

During the Phase II investigation, we will focus on a limited number of materials and applications. The top candidates will be selected, in part, based on the suitability of the candidate material for Raman analysis, as well as the economic significance of the application. A material is defined as being suitable if it evidences molecular compositional heterogeneity on a spatial scale that can be sampled with an optical microscope. In addition, the sample must not exhibit fluorescence that exceeds the dynamic range of the detector during the Raman imaging experiment. We have further selected target applications that represent sizable markets, in the likely event that benefits of LCTF Raman imaging are identified and demonstrated. Finally, the selection process is guided by the access to technical personnel within target organizations that recognize already the potential of LCTF Raman imaging. Dr. Treado has identified an extensive user base that have expressed considerable interest in the technology; these users have made real world samples available to the University of Pittsburgh so that a feasibility assessment can take place.

One very important application is in the non-invasive characterization of silicon semiconductors. Here, one would use LCTF Raman imaging to visualize the effect of ion implantation and thermal annealing on stress and polycrystalline distribution in the silicon lattice. The benefits of LCTF Raman imaging for this purpose can be assessed by comparing the quality and cost effectiveness of information collected by Raman imaging analysis with the information collected by competitive techniques. For example, lattice disorder and defects are currently monitored in silicon semiconductors using laser-scanning photoluminescence techniques. While this technique is sensitive to localized defects in semiconductors, photoluminescence is relatively nonspecific and does not provide sufficient information on the molecular composition of defect structures, whereas Raman would provide this compositional information. In addition, the impact of a localized defect or other heterostructure implanted in the Si lattice, can be monitored by

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visualizing the perturbation (stress or disorder) of the Si lattice
conformation.

This has value in the semiconductor and photonics device marketplace because
LCTF Raman imaging can be used for failure analysis and quality control. In
fact, there is significant potential that LCTF Raman imaging can evolve into
an in-line process monitoring tool to inspect potential failure sites in
highly integrated Si devices. The benefit would be a non-invasive approach to
monitor and reject devices at an intermediate stage of processing before many
thousands of dollars are invested in manufacturing high performance integrated
circuits or photonics devices (for example, CCD detectors) from these Si
wafers.

Additional applications include the following:
- Raman characterization of corrosion (bio-initiated or environment-
  initiated) occurring at microfractures in surfaces;
- thin film and coatings characterization, including the analysis of
  molecular compositional and conformational heterogeneity in polymer
  films;
- polymer blend domain structure analysis;
- content uniformity characterization of intact pharmaceutical tablets;
- oriented polymer fiber characterization;
- non-invasive analysis of combinatorial generated systems for
  pharmaceutical drug development; and,
- quantitative histopathology.

An important Government application is in exobiology, specifically the search
for life on Mars. NASA has expressed interest in an implementation of LCTF
Raman imaging technology for a Mars lander to monitor surface mineralogy, and
to screen for organic content that might suggest the past presence of life.
LCTF devices have already undergone successful environmental testing for
thermal/vacuum, flammability, and radiation load for such a mission[2]. Samples
provided by collaborators of Dr. Treado currently reside at the University of
Pittsburgh and are available for analysis.

v)   to make at least one of these LCTF instruments available to beta-site
      researchers for high-definition Raman imaging, on a revolving basis:

In Phase I and to date, essentially all the imaging Raman LCTF measurements of
real-world samples have been made by Dr. Patrick Treado at U/Pitt. During
Phase II, at least one Raman LCTF will be made available to other beta-site
researchers on a revolving basis. There are three reasons for this:

- it will provide valuable feedback from other leading Raman
  spectroscopists, who are perhaps more neutral in their assessment of
  this approach than the P.I., by virtue being disinterested in its
  success. Whether such feedback confirms or challenges our high
  expectations about the potential of the imaging LCTF Raman approach, a
  more complete picture of its utility will be obtained.

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- it will help to identify new applications, through testing of the filter in areas related to their individual research interests. As a new tool, the Raman LCTF will prove well-suited to some tasks and ill-suited to others. To find the matches requires insight into both the applications and the technology; putting these together requires collaboration with top researchers in a variety of areas.

- it will provide a scientifically valuable validation of the Phase I work, by other members of the community.

The Raman LCTF will be made available for a period of approximately 90 days, as progress and schedules warrant. As indicated in the Project Milestones, the filters will be completed well before the end of the first year, leaving considerable time for beta-site groups to use the filter. One such beta-site is already arranged with Dr. Desari of MIT's Harrison Spectroscopy Laboratory. He has agreed to work in this capacity in the Phase II effort, and has suitable laser, microscope, imaging, and computer equipment to make use the Raman LCTF. Additional beta-site users are being solicited, and we will seek to accommodate as many as is practical during the Phase II period.

vi)   to develop data analysis methods which exploit the newly-available imaging spectroscopic information, and which render the large data sets tractable;

There is a well-developed practice of Raman spectral analysis for point or spatially non-resolved data, including feature identification, principal component analysis, and quantitation of analyte species. These provide means to answer particular questions about the sample, such as what species are present, amounts, oxidation states, and so on. Such analyses can already be conducted on a pixel-by-pixel basis for the data sets collected using the LCTF Raman approach, using existing software packages.

However, our experience during the Phase I effort indicates that, while this type of analysis remains vital with Raman imaging data, it does not tell the whole story. To harvest the newly available spatial-spectral information, one wishes to answer new questions such as: what is the spatial distribution of each species, how do locations of two species correspond, are the structures co-incident or distinct, and similar queries. The answers often need to be displayed as high-resolution images rather than numerically. So there is a need, at a minimum, to integrate the analysis with improved display; that is, to permit various spectral-derived parameters to be calculated for each pixel, and then to display the derived parameters in image form.

This requirement defines a first level of data analysis which will be implemented in Phase II as a 'toolbox' of functions in a high-level interactive programming environment such as MatLab or ENVI, which is widely used in the remote sensing community for analysis of multi-spectral image cubes with two spatial dimensions and one spectral dimension. During the Phase II effort, we will discover what data specific tools are useful, and also which are compute-efficient to apply to a large data set. For this exploratory work, the desire for rapid feedback and interactive analysis is

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paramount, and exploration speed, rather than computational speed, is to be maximized. This goal would be met with a 'toolbox' approach in either of the proposed programming environments.

Based on the experience of the remote sensing community, there may also be a need for algorithms to sort through the large data sets and assess which spectral bands and features are informative, and which are not. If this is possible, it may lead to significant reduction in the size of the dataset and the processing time. Methods for this are presently used in the remote sensing community; their aptness for use in imaging Raman analysis will be assessed during Phase II. If promising, these algorithms or variants of them will form the basis of a second-level 'toolbox', to help extract the most meaning from the information which the Raman image cube provides.

G.   Commercial Potential
The clearest indicators of commercial potential are: C.R.I.'s demonstrated history of successes in commercializing SBIR developments; the presence of a Phase III partner who has committed to a follow-on funding agreement to gain access to the instruments being developed; and, a commercialization plan which seeks to exploit commercially the inherent potential of the LCTF Raman technology being developed.

G.1   C.R.I.'s demonstrated success in commercializing SBIR technology
Cambridge Research and Instrumentation, Inc., (CRI, Inc.) is a small (17 permanent employees) high-tech firm founded in 1985, with strengths in electro-optics and in space physics. Projected revenues in the current year are $3.2 M, of which about 70% is derived from commercial sales and the remainder from federal agency research contracts and grants. About 40% of the commercial sales consist of exports, primarily to Japan and W. Europe.

CRI's first (NSF SBIR-supported) product was a cryogenic absolute radiometer, for highest accuracy radiative flux measurements. The LaserRad and CryoRad-series radiometers, which sell for $75K - 150K, are now in use at national and aerospace metrology laboratories world-wide, as primary irradiance standards. A second successful product whose development was also supported in part by NASA and NSF SBIR awards, is an electro-optic servo system, priced between $4K - 8K, for control of laser power and removal of laser flux variations. The LPC-series stabilizers won two industrial awards in 1989, and CRI is the major world wide supplier of such systems into applications ranging from biomedical research to semi-conductor manufacturing. Cumulative sales in these two CRI products already exceed $5M, and annual sales continue to rise.

G.2   Phase III partnership and follow-on funding
A Phase III Follow-on Funding Commitment (FOFC) has been negotiated between C.R.I. and ChemIcon in the amount of $200,000, in return for first access to the technology being developed in Phase I and Phase II work. ChemIcon has already introduced a line of Raman microscopes based on the Phase I prototype, and have quotations pending with several customers totaling over $300K. They anticipate significantly larger markets as the improved Phase II devices become available, and as the utility of this new approach is assessed in key industrial applications.

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G.3   Commercialization Plan

The Phase III commercialization plan is attached in Appendix 2, and discusses the size of specific markets, competitive factors, patents, and other factors related to the commercial introduction of an LCTF Raman filter product.

H. Principal Investigator and Senior Personnel

PETER J. MILLER   PRINCIPAL INVESTIGATOR
Staff Scientist
Cambridge Research and Instrumentation, Inc.

Education:
        1980   B.A. (Astronomy)   Williams College
        1985   M.S. (Electrical Engineering)   Dartmouth College

Memberships:
        American Astronomical Society
        Optical Society of America

Experience:
1979   Visiting Fellow, JILA/NBS, Boulder, CO
1980-83   Staff Scientist, A.E.R., Inc., Cambridge, MA
1985   Staff Scientist; Cambridge Research and Instrumentation,
                     Inc., Cambridge, MA

Relevant Publications, Reports, and Awards
        "Liquid crystal tunable filter Raman chemical imaging", H. R. Morris, C.
        C. Hoyt, P. Miller, P. J. Treado, Appl. Spectroscopy 50 6, 805 (1996).

        "Liquid crystal tunable filters: theory and application to
        spectroscopy", P. J. Miller, FACSS Annual Meeting, (1996).

        "The development of a compact imaging spectrometer using liquid crystal
        tunable filter technology", J.A. Faust, A. Biswas, G. H. Bearman, T.
        Chrien, P. J. Miller, O.S.A. Annual Meeting (1996).

        "Multispectral imaging with a liquid crystal tunable filter", P. Miller,
        SPIE Proc. 2345, in press (1994).

        "Optical Retarder Having Means for Determining the Retardance of the
        Cell Corresponding to the Sensed Capacitance Thereof", U.S. Patent
        5,247,378 (1993).

        T. Chrien, C. Chovit, P. Miller, "Imaging spectrometry using a liquid
        crystal tunable filter", Proc. SPIE 1937, 257 (1993).

        R & D 100 Winners Announcement, Research & Development, 34, 12 (1992).
        Awarded for the VariSpec tunable liquid crystal filter.

        "Photonics Circle of Excellence Awards", Photonics Spectra, 26, 5
        (1992). Awarded for the VariSpec tunable liquid crystal filter.

        "Use of Tunable Liquid Crystal Filters to link Photometric and Radiomet-
        ric Standards", P. Miller, Metrologia 28, 145, (1991).

        "Liquid Crystal Devices and Systems Using Such Devices." P. Miller,
        U.S. Patent 4,848,877 (1989).

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I.   Consultants and Subawards

PATRICK J. TREADO

Assistant Professor   University of Pittsburgh   (1992 - present)
President   ChemIcon Inc.   (1994 - present)

EDUCATION:
1985   B.S. (Chemistry)   Georgetown University
1990   PH.D. (Analytical Chemistry) University of Michigan
1990-1992 POSTDOC (Biophysics)   National Institutes of Health

CONSULTING EXPERIENCE:
Cambridge Research & Instrumentation; Unilever; Westinghouse Electric
Corporation; Ford Motor Co.; Eli Lilly; Lockheed Martin; Proctor & Gamble;
Eastman Kodak; Medtronic; Johnson & Johnson; Bayer; PPG Industries

Prof. Treado is a recognized expert in the development of Raman microscopy and
its application to materials analysis. His letter confirming availability and
commitment to this project is included as an Appendix to this Proposal.

Prof. Treado's doctoral research involved the development of widefield Raman
microscopy techniques which led to a patent and several nationally competitive
fellowships and awards being won by Prof. Treado. As a postdoctoral fellow at
NIH, Prof. Treado pioneered the application of imaging spectrometers (acousto-
optic tunable filters and step-scan interferometers) to Raman, NIR and IR
imaging microscopy with Neil Lewis and Ira Levin. This work resulted in a
broad patent. In addition, papers describing the research received the 1993
and 1995 Spectroscopy for Applied Spectroscopy Meggers Awards.

Since joining the University of Pittsburgh, Department of Chemistry, Prof.
Treado has been engaged in the application of fluorescence, IR and Raman
chemical imaging to chemical analysis. In 1994, Prof. Treado founded ChemIcon
Inc., a company dedicated to commercializing chemical imaging technologies,
including Raman microprobes for continuous process monitoring, fluorescence
imaging systems to support drug discovery and Raman imaging microscopes.

Selected Publications

1.   Nicole K. Kline and Patrick J. Treado, Raman Chemical Imaging of Breast
     Tissue, J. Ram. Spectrosc. (1996) submitted.
2.   Michael D. Schaeberle, Costas G. Karakatsanis, Clifford J. Lau, and
     Patrick J. Treado, Raman Chemical Imaging: Histopathology of Inclusions
     in Human Breast Tissue, Anal. Chem. 68, (1996) 1829.
3.   Hannah R. Morris, Clifford C Hoyt, Peter Miller, and Patrick J. Treado,
     Liquid Crystal Tunable Filter (LCTF) Raman Chemical Imaging, Appl.
     Spectrosc. 50, (1996) 805.
4.   John F. Turner II and Patrick J. Treado, Near-Infrared Acousto-Optic
     Tunable Filter Hadamard Transform Spectroscopy, Appl. Spectrosc. 50,
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5. Nicole J. Kline and Patrick J. Treado, Raman Chemical Imaging of Disease
    States, *Proc. XVth ICORS*, S. A. Asher, Ed., (Wiley, Chichester, 1996)
    1190.
6. Hannah R. Morris and Patrick J. Treado, LCTF Raman Chemical Imaging of
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7. Michael D. Schaeberle and Patrick J. Treado, LCTF Raman Chemical Imaging
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    Chichester, 1996) 1188. ·
8. John F. Turner II and Patrick J. Treado, The Application of Chemometrics
    to Raman Chemical Imaging, *Proc. Xvth ICORS*, S. A. Asher, Ed., (Wiley,
    Chichester, 1996) 1202.
9. Michael D. Schaeberle, Costas G. Karakatsanis, Clifford J. Lau, and
    Patrick J. Treado, Raman Chemical Imaging: Noninvasive Visualization of
    Polymer Blend Architecture, *Anal. Chem.* 67, (1995) 4316.
10. E. Neil Lewis, Patrick J. Treado, Robert C. Reeder, Gloria M. Story,
    Anthony E. Dowrey, Curtis Marcott, Ira W. Levin, Fourier Transform Step-
    Scan Imaging Interferometry: High Definition Chemical Imaging in the
    Infrared Spectral Region, *Anal. Chem.* 67, (1995) 3377.
11. Patrick J. Treado, Chemical Imaging Reveals More Than the Microscope,
    Laser Focus World 31, (1995) 75.
12. Hannah R. Morris, Clifford C Hoyt, and Patrick J. Treado, Imaging
    Spectrometers for Fluorescence Microscopy: Acousto-Optic and Liquid
    Crystal Tunable Filters, *Appl. Spectrosc.* 48, (1994) 857.
13. Patrick J. Treado, Ira W. Levin, and E. Neil Lewis, Indium Antimonide
    (InSb) Focal Plane Array (FPA) Detection for Near-Infrared Imaging
    Microscopy, *Appl. Spectrosc.* 48, (1994) 607.
14. E. Neil Lewis, Patrick J. Treado, and Ira W. Levin, Near-Infrared and
    Raman Spectroscopic Imaging, *Amer. Lab.* 26, (1994) 16.
15. Patrick J. Treado and Michael D. Morris, Infrared and Raman Spectroscopic
    Imaging, *Spectroscopic and Microscopic Imaging of the Chemical State*,
    M.D. Morris, Ed. (Marcell Dekker, New York, 1992) pp. 71-108.
16. Patrick J. Treado, Ira W. Levin and E. Neil Lewis, High-Fidelity Raman
    Imaging Spectrometry: A Rapid Method Using an Acousto-optic Tunable ·
    Filter, *Appl. Spectrosc.* 46, (1992) 1211.
17. Patrick J. Treado and Michael D. Morris, Multichannel Hadamard Transform
    Raman Microscopy, *Appl. Spectrosc.* 44, (1990) 1.

J.   Equipment, Instrumentation, Computers, and Facilities
J.1   Facilities available at C.R.I.
Of particular importance to this project is the C.R.I. liquid crystal filter
fabrication facility. This includes an 8 x 12 foot class-100 clean room with
complete thermal and humidity control; a 12 x 24 foot class-1000 clean room
with complete thermal and humidity control; all necessary equipment for
fabricating liquid crystal elements of the highest optical quality on glass
and quartz substrates, including a semiconductor-class wash line, photoresist
and etch process lines, spin-coating equipment, spacer-deposition chambers,
ovens (atmospheric and vacuum), DI water filtration units, desiccant storage
vaults, along with equipment for buffing, assembly and filling of liquid
crystal cells. There are several class 100 horizontal flow hoods located
within the class 1000 space, for overall filter assembly in a particle-free,
laminar flow environment. This facility, staffed by two technicians and a
senior process engineer, produces liquid crystal cells of unsurpassed optical
quality and transmission.

Test equipment at C.R.I. includes a 0.5-meter SPEX spectrometer with 300 and
1200 line/mm gratings for high-resolution spectroscopy (0.025 nm); a quarter-
meter visible ISA spectrometer with an EG&G 1024-element reticon detector; a
Scitec optical chopper and Stanford Research 810 lock-in amplifier; quartz-
halogen, mercury, Kanthal, and Xe arc continuum sources with collimation
optics; a mercury line source for wavelength scale calibration; and a variety
of silicon, germanium, PbSe, and InGaAs photodiodes, and photodiode preamps
with NIST-traceable calibration. General optical equipment includes a 4' x 8'
air-suspended optical table, a Neslab RTD-110 controlled temperature system
with heat sink mount for temperature control of optical samples to +/- 0.02
°C; He-Ne, Ar^{++}, and Nd:YAG lasers with spatial mode filters and beam expan-
ding optics; 6-inch and 1-inch Labsphere integrating spheres; PC/pentium
computers with high-speed data acquisition hardware and software.

Imaging equipment includes a Zeiss Axioskop microscope equipped for epi- and
kohler illumination; a Dage MTI CCD-72 camera with TEC-1 cooler, single-line
gate, and controller; a Matrox MVP-AT frame grabber; Optimus, MetaMorph, and
ImagePro image processing software; and a Sony Trinitron RGB monitor. C.R.I.
maintains a network of PC/486 and PC/pentium computers with printers, tape
drives, B and C size plotters, and Internet access. MatLab and ENVI software
are available for the data reduction tasks described in the Plan Of Work.

In addition, C.R.I. has a PC/pentium-based CAD center for electronic and
mechanical design using AutoCAD, PowerPCB, and ViewLogic design tools; a
software development lab for programming the Intel 8031 and 80196 family of
embedded microcontrollers; and an electronics test and production lab
equipped to handle analog, digital, and microprocessor assemblies. We have
access to complete metal-working facilities for fabricating jigs, special
optical fixtures, and the like.

J.2   Facilities available to Prof. Treado at the University of Pittsburgh
Laboratory Facilities:
Prof. Treado has approximately 1200 ft^2 of modern laboratory space, equipped
for analytical spectroscopy (visible/NIR, Raman and fluorescence), optical

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microscopy, scanning electron microscopy (SEM), wet chemistry and sample preparation.

Computer:
Data collection is controlled by PCs (Pentiums and 486s) running commercial programs, including ChemImage, a Windows program for multispectral image visualization and analysis. The PCs are networked to a Silicon Graphics IRIS Indigo R4K workstation fileserver with mass data storage, backup and retrieval. The workstation serves as an advanced spectral image processing workstation using MATLAB and ENVI.

Major equipment in Prof. Treado's laboratory:
(2) Princeton Instruments CCD detectors interfaced to a Gateway 2000 50 MHz 80486 DX2 computer running BioScan OPTIMAS image processing software; Coherent 330 Krypton ion laser, 400 mW 752 nm, 1 W multiline red; Coherent CR3 Argon ion laser, 150 mW 514.5 nm; (2) Chromex 0.5 m spectrographs for dispersive optical spectroscopy, and spectral calibration of the LCTFs; (2) Olympus BH2 upright microscopes interfaced to Cohu 4910 b&w video cameras for sample positioning and focusing; Kinetic Systems research grade optical table, 4' x 10'; Newport research grade optical table, 5' x 10'; EG&G PAR 5110 lock-in amplifier; E-Tec scanning electron microscope; Electro-Optic Systems InSb photodiode; CRI VariSpec LCTF for fluorescence imaging and for Raman imaging; Scanalytics CellScan Numerical Deconvolution Confocal microscopy software.

Industrial materials:
Sophisticated samples suitable for Raman microscopy applications including Martian meteorites, corrosion samples (steam-generator components), polymeric composites, thin films and coatings, silicon semiconductors, combinatorial substrates, and microelectrodes, as well as pharmaceuticals, will be provided by Prof. Treado's external collaborators in the defense, pharmaceutical, semiconductor and polymer industries.

Other:
The chemistry department maintains the usual array of modern spectroscopic instrumentation available for general access. Fully equipped electronics and machine shops for instrument and prototype fabrication are available.

K.   Current and Pending Support of P.I. and Senior Personnel
The P.I. has no current or pending support for development of Raman imaging filters. He has a commitment of 1.5 months to N.I.H. contract #2 R44 MH53690-02, entitled "High-efficiency Tuneable Fluorescence Emission Filter", during the first year of the Phase II period of performance. Further commitments to commercially-sponsored liquid-crystal tunable filter development contracts are approximately 25% at present, and this level is anticipated during Phase II.

L.   Equivalent or Overlapping Proposals to Other Federal Agencies
There are no equivalent proposals pending for development of Raman imaging filters, nor any which overlap the present Plan of Work.

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Year 1 Budget

APPENDIX D

(SEE INSTRUCTIONS ON REVERSE BEFORE COMPLETING)	PROPOSAL BUDGET		FOR NSF USE ONLY	
ORGANIZATION Cambridge Research & Instrumentation, Inc.			**PROPOSAL NO.** DMI-9560600	**DURATION (MONTHS)** Proposed / Granted
PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR Peter J. Miller			**AWARD NO.**	24

A. SENIOR PERSONNEL: PI/PD and Other Senior Associates (List each separately with title, A.8, show number in brackets)	NSF Funded Person-mos. CAL	Funds Requested By Proposer	Funds Granted By NSF (if Different)
1. P.I.	4.6	$ 28,000	$
2.			
3.			
4.			
5.			
6. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET EXPLANATION PAGE)			
7. ( ) TOTAL SENIOR PERSONNEL (1-5)			
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)			
1. ( ) POST DOCTORAL ASSOCIATES			
2. (2) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	2.2	7,000	
3. ( ) GRADUATE STUDENTS			
4. ( ) UNDERGRADUATE STUDENTS			
5. ( ) SECRETARIAL - CLERICAL			
6. ( ) OTHER			
TOTAL SALARIES AND WAGES (A+B)		35,000	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS) 0.38 x (A+B)		13,300	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A+B+C)		48,300	
D. PERMANENT EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.) see list			
TOTAL PERMANENT EQUIPMENT		39,600	
E. TRAVEL 1. DOMESTIC (INCL. CANADA AND U.S. POSSESSIONS		2,000	
2. FOREIGN (Do not use for Phase I)		—	
F. PARTICIPANT SUPPORT COSTS			
1. STIPENDS $_____			
2. TRAVEL _____			
3. SUBSISTENCE _____			
4. OTHER _____			
( ) TOTAL PARTICIPANT COSTS			
G. OTHER DIRECT COSTS			
1. MATERIALS AND SUPPLIES (Attach itemized list if over $5,000) LCTF materials, see list		10,000	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION		1,000	
3. CONSULTANT SERVICES (Attach confirmation letters) (Daily rate not over $443)		14,000	
4. COMPUTER (ADPE) SERVICES		1,000	
5. SUBCONTRACTS Univ. Pittsburgh (grad student, 3.5 mos.)		9,000	
6. OTHER			
TOTAL OTHER DIRECT COSTS			
H. TOTAL DIRECT COSTS (A THROUGH G)		124,900	
I. INDIRECT COSTS (SPECIFY) Overhead = 0.46 x (A+B+C) =22,218 TOTAL INDIRECT COSTS G&A = 0.16 x (H+overhead) =23,539		45,757	
J. TOTAL DIRECT AND INDIRECT COSTS (H+I)		170,656	
K. FEE (if requested; maximum equals 7% of J)		9,786	
L. TOTAL COST AND FEE (J + K)		$180,442	$

PI/PD TYPED NAME & SIGNATURE Peter J. Miller	DATE 10/23/96	FOR NSF USE ONLY		
		INDIRECT COST RATE VERIFICATION		
CO. REP. TYPED NAME & SIGNATURE Peter V. Foukal	DATE 10/24/96	Date Checked	Date of Rate Sheet	Initials-DGA

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Year 2 Budget

APPENDIX D

(SEE INSTRUCTIONS ON REVERSE BEFORE COMPLETING)	PROPOSAL BUDGET		FOR NSF USE ONLY	
ORGANIZATION			PROPOSAL NO.	DURATION (MONTHS)
Cambridge Research & Instrumentation			DMT-9560600	Proposed / Granted
PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR			AWARD NO.	
Peter J. Miller				24

A. SENIOR PERSONNEL: PI/PD and Other Senior Associates (List each separately with title, A.6, show number in brackets)	NSF Funded Person-mos. CAL	Funds Requested By Proposer	Funds Granted By NSF (If Different)
1. P. T.	4.4	$26,000	$
2.			
3.			
4.			
5.			
6. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET EXPLANATION PAGE)			
7. ( ) TOTAL SENIOR PERSONNEL (1-6)			
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)			
1. ( ) POST DOCTORAL ASSOCIATES			
2. (2) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	1.8	6,000	
3. ( ) GRADUATE STUDENTS			
4. ( ) UNDERGRADUATE STUDENTS			
5. ( ) SECRETARIAL - CLERICAL			
6. ( ) OTHER			
TOTAL SALARIES AND WAGES (A+B)		32,000	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS) 0.38 x (A+B)		12,160	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A+B+C)		44,160	
D. PERMANENT EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.) see list			
TOTAL PERMANENT EQUIPMENT		3,000	
E. TRAVEL 1. DOMESTIC (INCL. CANADA AND U.S. POSSESSIONS		3,500	
2. FOREIGN (Do not use for Phase 1)		—	
F. PARTICIPANT SUPPORT COSTS			
1. STIPENDS $			
2. TRAVEL			
3. SUBSISTENCE			
4. OTHER			
( ) TOTAL PARTICIPANT COSTS		—	
G. OTHER DIRECT COSTS			
1. MATERIALS AND SUPPLIES (Attach itemized list if over $5,000)		—	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION		1,500	
3. CONSULTANT SERVICES (Attach confirmation letters) (Daily rate not over $443)		14,000	
4. COMPUTER (ADPE) SERVICES		2,000	
5. SUBCONTRACTS Univ. Pittsburgh(grad student 3.5 mos)		9,000	
6. OTHER			
TOTAL OTHER DIRECT COSTS			
H. TOTAL DIRECT COSTS (A THROUGH G)		77,160	
I. INDIRECT COSTS (SPECIFY) overhead = 0.46 x (A+B+C) = 20,314			
TOTAL INDIRECT COSTS G&A = 0.16 x (H+overhead) =15,596		35,910	
J. TOTAL DIRECT AND INDIRECT COSTS (H+I)		113,070	
K. FEE (If requested; maximum equals 7% of J)		6,488	
L. TOTAL COST AND FEE (J + K)		$119,558	$

PI/PD TYPED NAME & SIGNATURE	DATE	FOR NSF USE ONLY		
Peter J. Miller	10/23/96	INDIRECT COST RATE VERIFICATION		
CO. REP. TYPED NAME & SIGNATURE	DATE	Date Checked	Date of Rate Sheet	Initials-DGA
Peter V. Foukal	10/24/96			

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Summary Budget

APPENDIX D

(SEE INSTRUCTIONS ON REVERSE BEFORE COMPLETING)	PROPOSAL BUDGET			FOR NSF USE ONLY	
ORGANIZATION Cambridge Research & Instrumentation, Inc.				**PROPOSAL NO.** DMI-9560600	**DURATION (MONTHS)** Proposed / Granted
PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR Peter J. Miller				**AWARD NO.**	24

A. SENIOR PERSONNEL: PI/PD and Other Senior Associates (List each separately with title. A.6. show number in brackets)		NSF Funded Person-mos. CAL		Funds Requested By Proposer	Funds Granted By NSF (If Different)
1. P.I.		9		$ 54,000	$
2.					
3.					
4.					
5.					
6. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET EXPLANATION PAGE)					
7. ( ) TOTAL SENIOR PERSONNEL (1-5)					
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. ( ) POST DOCTORAL ASSOCIATES					
2. (2) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)		4		13,000	
3. ( ) GRADUATE STUDENTS					
4. ( ) UNDERGRADUATE STUDENTS					
5. ( ) SECRETARIAL - CLERICAL					
6. ( ) OTHER					
TOTAL SALARIES AND WAGES (A-B)				67,000	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				25,460	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A+B+C)				92,460	
D. PERMANENT EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.) see list					
TOTAL PERMANENT EQUIPMENT				42,600	
E. TRAVEL 1. DOMESTIC (INCL. CANADA AND U.S. POSSESSIONS				5,500	
2. FOREIGN (Do not use for Phase I)				—	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $					
2. TRAVEL					
3. SUBSISTENCE					
4. OTHER					
( ) TOTAL PARTICIPANT COSTS					
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES (Attach itemized list if over $5,000) LCTF Supplies - see list				10,000	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				2,500	
3. CONSULTANT SERVICES (Attach confirmation letters) (Daily rate not over $443)				28,000	
4. COMPUTER (ADPE) SERVICES				3,000	
5. SUBCONTRACTS Univ. Pittsburgh (grad student, 7 months)				18,000	
6. OTHER					
TOTAL OTHER DIRECT COSTS					
H. TOTAL DIRECT COSTS (A THROUGH G)				202,060	
I. INDIRECT COSTS (SPECIFY) Overhead 0.46 x (A+B+C) =42,532					
TOTAL INDIRECT COSTS G&A 0.16 x (H+overhead)=39,135				81,667	
J. TOTAL DIRECT AND INDIRECT COSTS (H+I)				283,726	
K. FEE (If requested; maximum equals 7% of J)				16,274	
L. TOTAL COST AND FEE (J + K)				$300,000	$

PI/PD TYPED NAME & SIGNATURE Peter J. Miller	DATE 10/23/96	FOR NSF USE ONLY		
		INDIRECT COST RATE VERIFICATION		
CO. REP. TYPED NAME & SIGNATURE Peter V. Foukal	DATE 10/23/96	Date Checked	Date of Rate Sheet	Initials-DGA

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M.1   Justification of Permanent Equipment Items

Year 1
    Spectrometers (Kaiser HoloSpec f/2.2 HFG-650, HFG-850) $11,600
    CCD (Princeton Instr. STC-133/RTE-CCD-64-H) $ 9,000
    LiNbO$_3$ substrate blanks (64 @ $125/ea.) $ 8,000
    Polishing of LiNbO$_3$ substrates (64 @ $125/ea.) $ 8,000
    Holographic optical elements (3 @ $1,000/ea) $ 3,000

Year 2
    Holographic optical elements (3 @ $1,000/ea) $ 3,000

The spectrometers will be used to characterize the retarder components and filters used in this work. Existing equipment used for this work is the Spex 0.5M spectrometer. This is a mechanically-scanned instrument, and it is not possible to obtain wide spectral coverage by outfitting it with a CCD array detector. The time to acquire a spectrum is so long (20 minutes) that characterization of important parameters required herein, such as thermal drift and off-axis response, become impractical. In contrast, the Kaiser instruments use a transmissive holographic grating to image the entire working range onto a 25 mm CCD array detector. Reliability is improved relative to the present equipment, by removing the moving parts; this is especially important since present equipment requires unattended operation for days at a time. More important, data acquisition time is reduced by a factor of >100 due to parallel data collection at the CCD, making the Plan of Work practical.

The Kaiser grating HFG-650 covers the range 500 - 800 nm, and the NIR grating HFG-850 spans 655 - 1045 nm, with a resolution of 10 cm^{-1} or better at a 1024-element CCD. Due to the pricing of the Kaiser components, there is only a modest premium (16%) for buying two spectrometers, compared to one spectrometer with two gratings. This is a transmissive spectrometer, with no provision for a rotating turret to switch between various gratings. For this reason, and to minimize the risk of damaging the valuable gratings in handling, the purchase of two spectrometers is proposed.

The CCD will be used in concert with the spectrometers to obtain spectral data on the retarders and assembled LCTF, not for acquisition of Raman images. Coupling is by means of a rugged, detachable adapter so one CCD can be readily used with both spectrometers. Princeton Instruments has expressed an interest in the imaging LCTF Raman technology, and has agreed to provide a model STC-133 camera with RTE/CCD-64-H readout electronics at a significantly reduced price ($9,000 vs. $12,980 list) in order to learn more about the application. Thermoelectric cooling of the CCD array is sufficient, as even the most demanding tests (such as the assessment of spectral leakage in completed filters) require exposures of only a few seconds.

The LiNbO$_3$ blanks, polished to form precision waveplates, are used to build the visible and NIR prototypes, as per Objective iii). Based on the Phase I experience, we anticipate that 64 LiNbO$_3$ blanks (of selected thicknesses) will yield between 40 - 50 good parts; 32 are required to complete the Plan of Work.

Holographic optical elements (HOEs) are used in the Raman imaging microscope
to block Rayleigh scatter at the laser wavelength. There are existing HOE's
at U/Pitt for the wavelengths up to 647 nm; however, with the development of
the NIR filter, we intend to operate at new, longer wavelengths up to at least
780 nm. Each wavelength requires a dedicated HOE, and this budget item will
be used to procure these, for use at U/Pitt and at beta-sites in Year 2.

M.2   Justification of travel expenses
Travel between Cambridge and U/Pitt by the P.I. or Dr. Treado is anticipated,
and is budgeted as 2 round-trip visits per year during the project at $500 per
visit. Conference travel is budgeted as $1,000 per year. In the second year
the P.I. plans to visit the beta-site laboratories; one is local (M.I.T.),
but others are expected to require airfare and lodging costs.

M.3   Justification of materials costs
Year 1
    Materials
    NIR polarizers (32 @ $125 each) $ 4,000
    liquid crystal material, substrates, epoxies, etc. $ 4,000
    optical coating charges (4 lots @ $500 each) $ 2,000

Year 2
    (none)

All supplies will be used to construct the Phase II prototypes. Coating
charges are for anti-reflection coatings in the NIR and visible, which are
deposited on $LiNbO_3$ and on glass; a total of four coatings lots are required.

M.4   Justification of Consultant and Subcontract services
Consulting funds of $28K are budgeted for Dr. Treado to perform his portion of
the work at U/Pitt. Dr. Treado will be principally responsible for the task
of meeting objective iv), the evaluation of applications targeted as having
commercial potential. This is a laboratory research task, involving imaging
Raman analysis of various samples relevant to the target applications.
Initially, Dr. Treado will use the Phase I prototype, and this will be
replaced with improved Phase II parts as they become available. He will be
assisted in this work by a graduate student at the University.

Dr. Treado's rate for this work is $442 per day, and an effort equivalent to
three calendar months (62 working days) is budgeted during the 2-year Phase II
period of performance. Seven months of graduate student support is included
as well, through a subcontract to the Univ. of Pittsburgh for $2.6K per month
of student support; this includes their overhead, and a lesser amount is paid
the student. A letter from the University is attached to this Proposal,
indicating the terms of this arrangement.

References
1.   J. W. Evans, "The Birefringent Filter", J. O.S.A. 39, 3, 229 (1949).
2.   J.A. Faust, A. Biswas, G.H. Bearman, T. Chrien. P.J. Miller,
      "Development of a compact imaging spectrometer using liquid crystal
      tunable filter technology", O.S.A. 1996 Annual meeting, talk ThII7
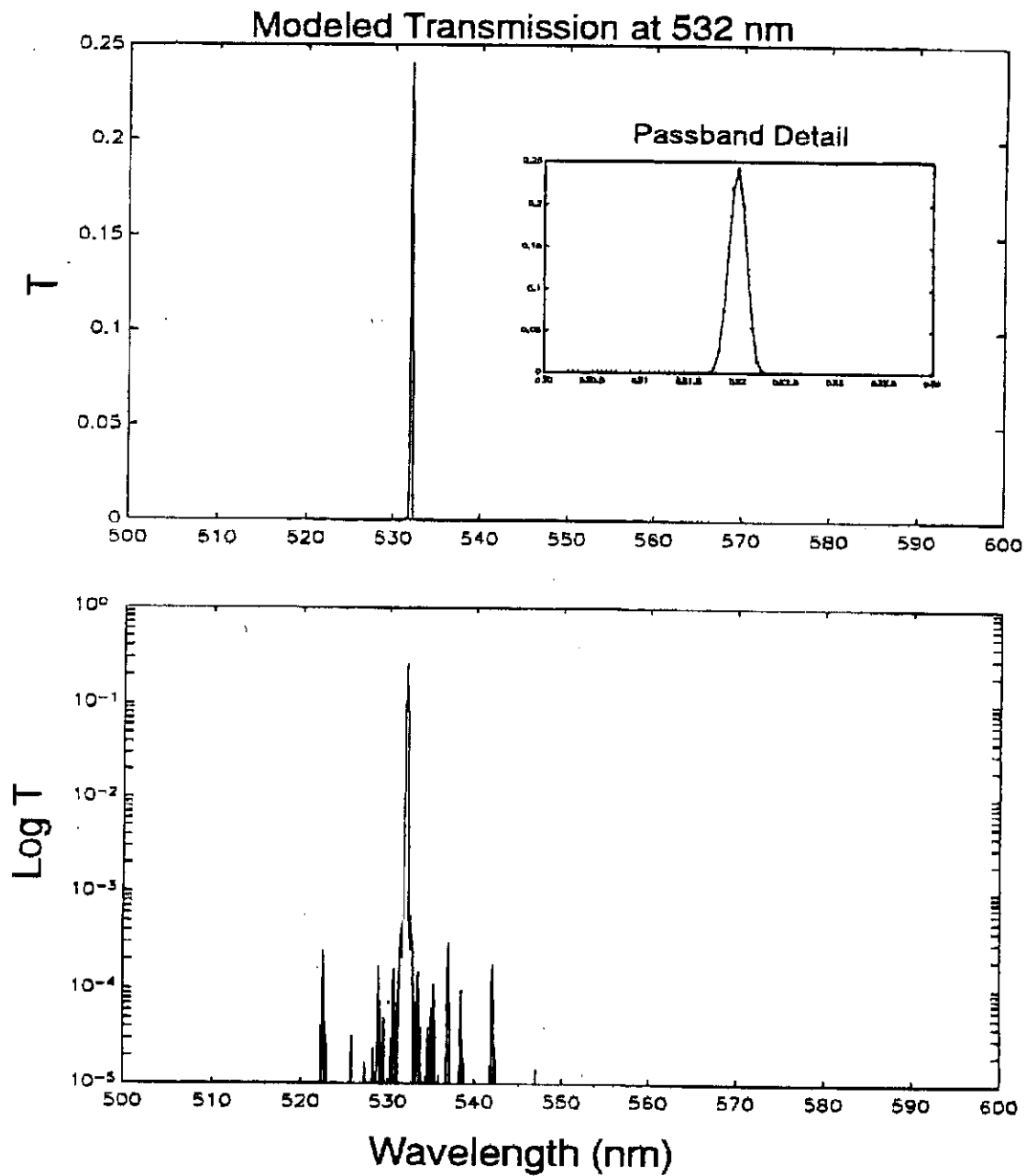   (1996).

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Figure 1. Model LCTF performance when tuned to 532 nm, shown on linear and logarithmic scales. Passband detail is shown in the inset.
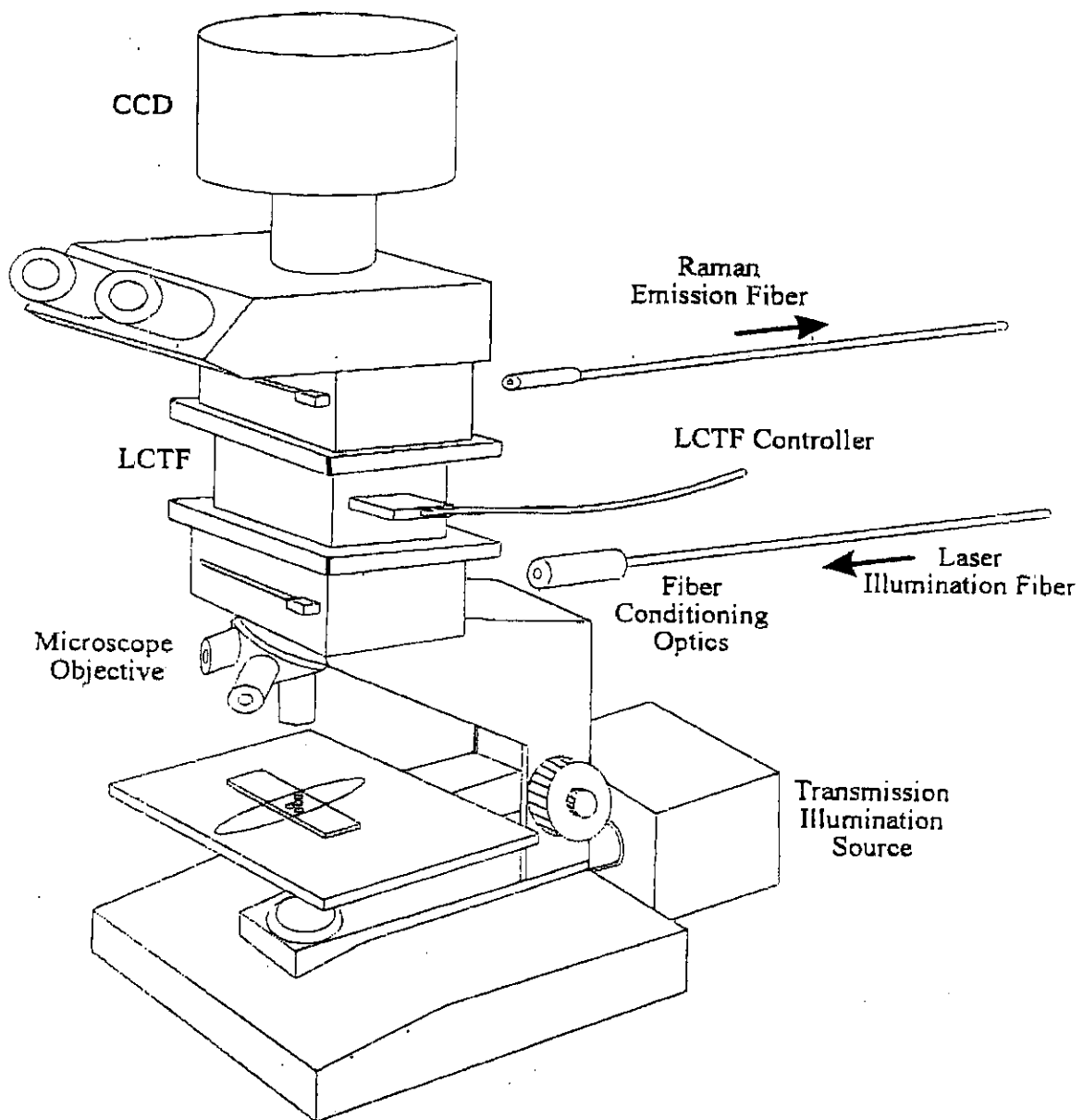
Figure 2. Diagram of the LCTF Raman filter integrated onto the optical microscope.
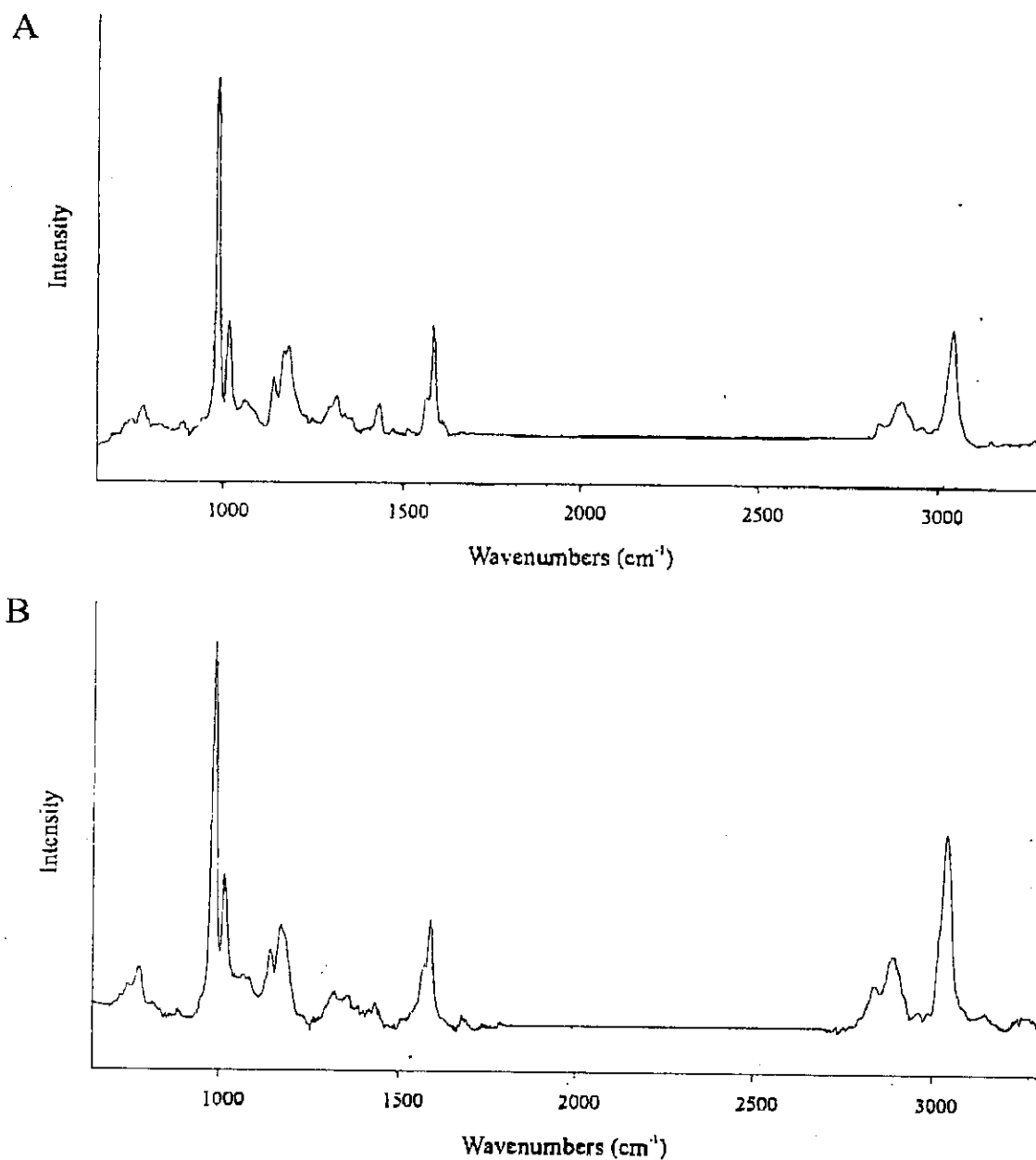
27

Figure 3. The top figure shows a Raman spectrum of polystyrene microsphere, obtained with the LCTF, while the lower figure shows the Raman spectrum of the same sample taken through a conventional (non-imaging) dispersive spectrometer system.
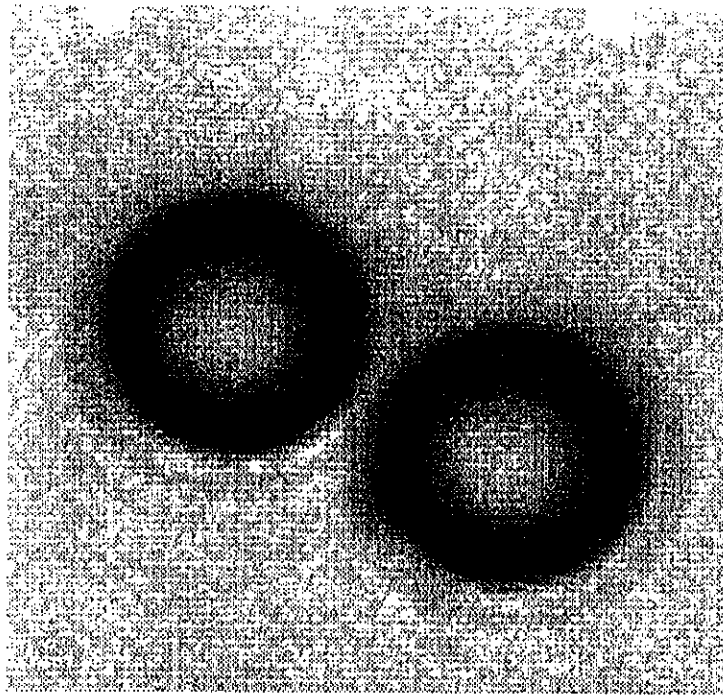
28

Figure 4. Diffraction-limited image of 2 μm polystyrene spheres (brightfield).

Figure 5. Raman image at 992 cm-1 of the same sample. Note 500 nm size bar shown for reference.

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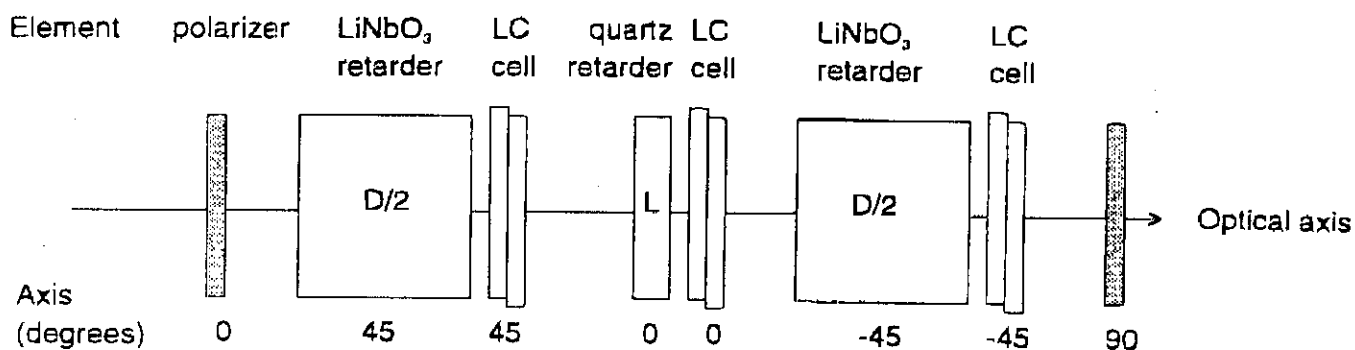| Element	polarizer	LiNbO₃ retarder	LC cell	quartz retarder	LC cell	LiNbO₃ retarder	LC cell	

| Axis (degrees) | 0 | 45 | 45 | 0 | 0 | -45 | -45 | 90 |

Figure 6. Diagram of an Evans split element stage. Thicknesses and spacing between elements is greatly exaggerated for clarity. Axes indicated are the transmission axis for polarizers, and the fast axis for LC cells and retarders.
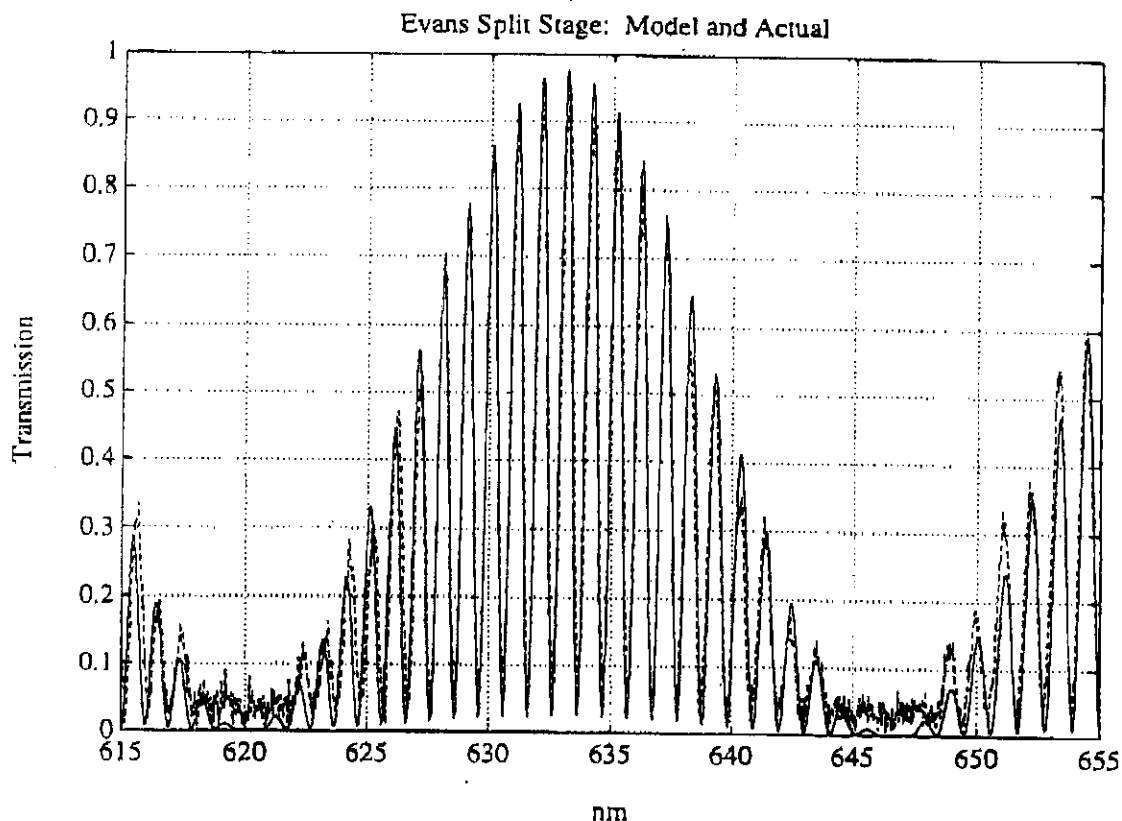

Figure 7. Transmission of a single Evans split element stage, constructed in Phase I, along with model predictions. The high-frequency structure is produced by the D/2 retarders, and the envelope is generated by the R_L quartz retarder.

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CERTIFICATE OF CURRENT COST OR PRICING DATA

This is to certify that, to the best of my knowledge and belief, the cost or pricing data (as defined in section 15.801 of the Federal Acquisition Regulations), submitted either actually or by specific identification in writing, to the Grant Officer or to the Grants Officer's representative in support of ___DMI-9560600_____ * are accurate, complete, and current as of ___10/17/96_____.**

This certification includes the cost or pricing data supporting any advance agreements and forward pricing rate agreements between the offeror and the Government that are part of the proposal.

COMPANY NAME: Cambridge Research & Instrumentation, Inc.

REPRESENTATIVE NAME:___Peter V. Foukal_____

REPRESENTATIVE TITLE:___President_____

REPRESENTATIVE
    SIGNATURE:___*[signature]*_____

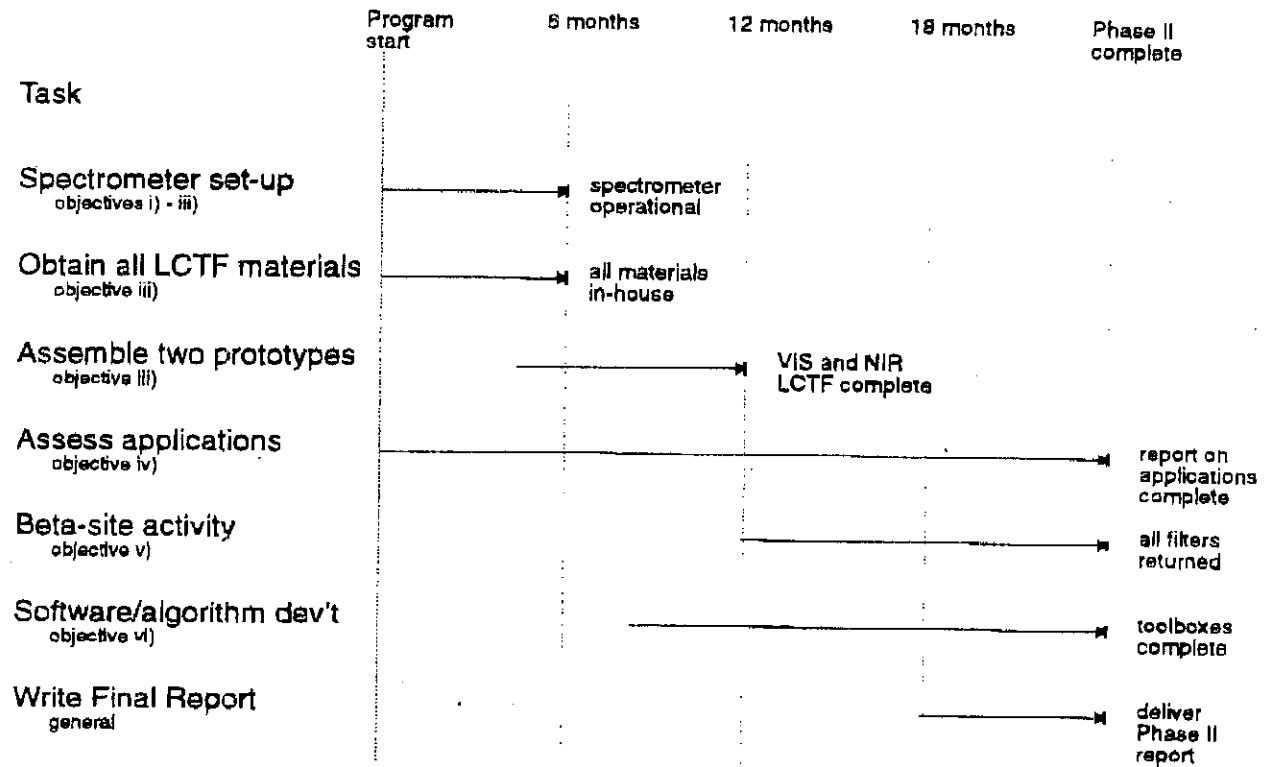DATE OF
    EXECUTION***:___10/17/96_____

SBIR Organizations are required to submit this certificate with their proposal. If the proposal is recommended for funding, a second certificate will be requested by NSF subsequent to a pre-award budget review, but prior to award.

" Identify the SBIR Phase II Proposal Number.

** (1) Insert the date, month, and year for proposal submission when submitting with proposal, (2) or the date, month, and year when notified by NSF that the proposal has been recommend for award and price negotiations are completed.

*** Insert the date, month, and year of signing

Appendix 1. SBIR Phase II Milestone chart

	Program start	6 months	12 months	18 months	Phase II complete

Task

Spectrometer set-up
objectives i) - iii) → spectrometer operational

Obtain all LCTF materials
objective iii) → all materials in-house

Assemble two prototypes
objective iii) → VIS and NIR LCTF complete

Assess applications
objective iv) → report on applications complete

Beta-site activity
objective v) → all filters returned

Software/algorithm dev't
objective vi) → toolboxes complete

Write Final Report
general → deliver Phase II report

Effort (months)	1-6	7-12	13-18	19-24
Key personnel	2.60	2.00	2.00	2.40
Consultants	0.25	0.25	0.25	0.25
Subcontracts	1.75	1.75	1.75	1.75

Estimated Expenditures				
Key personnel	21,520	16,560	16,560	19,872
Consultants	7,000	7,000	7,000	7,000
Subcontracts	4,500	4,500	4,500	4,500
Perm. Equip.	36,600	3,000	3,000	-
Materials	10,000	-	-	-
Other	38,547	21,429	24,172	26,466

<u>Appendix 2A: Indicators of Commercial Potential</u>

The clearest indicators of commercial potential are: C.R.I.'s demonstrated
history of successes in commercializing SBIR developments; the presence of a
Phase III partner who has committed to a follow-on funding agreement to gain
access to the instruments being developed; and, a commercialization plan
which seeks to exploit commercially the inherent potential of the LCTF Raman
technology being developed.

C.R.I.'s demonstrated success in commercializing SBIR technology
Cambridge Research and Instrumentation, Inc., (CRI, Inc.) is a small (17
permanent employees) high-tech firm founded in 1985, with strengths in
electro-optics and in space physics. Projected revenues in the current year
are $3.2 M, of which about 70% is derived from commercial sales and the
remainder from federal agency research contracts and grants. About 40% of the
commercial sales consist of exports, primarily to Japan and W. Europe.

CRI's first (NSF SBIR-supported) product was a cryogenic absolute radiometer,
for highest accuracy radiative flux measurements. The LaserRad and CryoRad-
series radiometers, which sell for $75K - 150K, are now in use at national and
aerospace metrology laboratories world-wide, as primary irradiance standards.
A second successful product whose development was also supported in part by
NASA and NSF SBIR awards, is an electro-optic servo system, priced between $4K
- 8K, for control of laser power and removal of laser flux variations. The
LPC-series stabilizers won two industrial awards in 1989, and CRI is the major
world wide supplier of such systems into applications ranging from biomedical
research to semi-conductor manufacturing. Cumulative sales in these two CRI
products already exceed $5M, and annual sales continue to rise.

Phase III partnership and follow-on funding
A Phase III Follow-on Funding Commitment (FOFC) has been negotiated between
C.R.I. and ChemIcon in the amount of $200,000, in return for first access to
the technology being developed in Phase I and Phase II work. The FOFC is
attached as an Appendix to the Proposal. ChemIcon has already introduced a
line of Raman microscopes based on the Phase I prototype, and have quotations
pending with several customers totaling over $300K. They anticipate
significantly larger markets as the improved Phase II devices become
available, and the utility of this new approach is assessed in key industrial
applications.

Commercialization plant to exploit LCTF Raman technology
The commercialization plan follows, and outlines the product, company,
competitors, and marketing and production plans for the Phase III (commercial)
phase of this project.

Appendix 2.B   Commercialization Plan

Company
Cambridge Research & Instrumentation, Inc., was founded by Dr. Peter Foukal in
1985 to perform solar-terrestrial physics research, and to develop scientific
equipment related to the precision measurement and control of light. Among
its products are the Laser Power Controller, which won the R&D 100 and
Photonics Circle of Excellence awards when it was introduced in 1989, and
which is widely used today in research and in semiconductor wafer marking;
the LaseRad and CryoRad cryogenic absolute radiometers, which are used as the
primary standard of light measurement at NIST, as well as at the National
laboratories in Germany, Canada, Sweden, the Netherlands, Spain, and China;
and the VariSPEC liquid crystal tunable filters. The VariSPEC line has
received great commercial success, also received numerous awards, including
the R&D 100, Laser Focus World CTA, and Photonics Circle of Excellence.

C.R.I. has 18 employees, is privately held, and has been profitable every year
since its formation. Sales for the current fiscal year are approximately
$3.2M, of which approximately 30% are overseas exports. C.R.I. has been
awarded patents for liquid crystal components related to LCTF construction,
and holds exclusive license to others, including key patents on RGB color
filters and a polarization microscope system developed by the Marine
Biological Laboratory at Woods Hole.

SBIR Project
This SBIR project seeks to exploit narrowband tunable filters, similar to
C.R.I.'s existing line of VariSPEC filters, to take images of the Raman
emission of samples in microscopes. In this way, the chemical 'fingerprint'
of the sample is obtained with great specificity. There is essentially no
sample preparation, and the measurement is nondestructive. The information
which is obtained provides a highly detailed picture, or image, of the
location and quantities of different chemical species in the sample.

Such highly detailed imaging information is otherwise difficult or impossible
to obtain, and provides a wealth of information on heterogeneous samples such
as patterned silicon wafers, cellular tissue, thin films, and a variety of
real-world items which have structures within them.

By making and optimizing the filters used in this approach, and then testing
the overall imaging system in key target applications, C.R.I. seeks to
identify suitable markets for this new instrument.

Commercial Applications
Applications for a Raman imaging microscope are diverse because almost every
manufactured or natural material has a unique, intrinsic Raman spectral
fingerprint. The Raman spectra can be harnessed to generate molecule-specific
image contrast that is not derived from time consuming, potentially invasive
sample staining processes. As a result almost every material analysis
application can potentially benefit from Raman imaging analysis. The broad
applicability of this technique is one of the attractive features of Raman
imaging.

The major criteria for applicability are that the material of interest
evidence molecular chemical heterogeneity on a spatial scale that can be

probed by an optical microscope (>200 nm) and that the heterogeneity be stable on the time scale of a typical Raman imaging analysis (mins.); a broad range of materials and potential applications fall within these guidelines.

Prof. Treado's research at the University of Pittsburgh has been focused in the past several years on identifying the applications that derive the most benefit from Raman image analysis. A partial list of applications follows:

- non-invasive characterization of ion implantation and thermal annealing on silicon semiconductors;
- characterization of phosphate-initiated corrosion in steam generator components;
- analysis of bio-initiated corrosion in implantable medical devices;
- thin film and coatings characterization, including analysis of molecular compositional and conformational heterogeneity in diamond films, polymer films, and ferroelectric thin films;
- polymer blend domain structure analysis;
- content uniformity characterization and polymorphism content analysis of intact pharmaceutical tablets;
- oriented polymer fiber characterization;
- adhesion characteristics of thin polymer films;
- surface crystallinity content analysis of controlled-release polymer drug delivery systems;
- non-invasive analysis of combinatorial systems employed in biosensors and in drug discovery;
- quantitative histopathology;
- the search for life on Mars with the implementation of LCTF Raman imaging technology in a Mars lander.

Patent Status
C.R.I. holds two key patents related to the construction of liquid crystal tunable filters (LCTFs), and another is pending. These protect the core technology, including the work described in the present SBIR proposal. No patents have been filed as a result of the Phase 1 effort, but it is anticipated that patentable work may result from the Phase II project and a periodic evaluation of the patentability of the research will be performed and acted upon where appropriate.

Innovation
Compared to existing, non-imaging systems, the Raman LCTF system adds the powerful ability to visualize the distribution (morphology and architecture) of chemical species in heterogeneous samples with molecular compositional specificity. Raman images can be collected rapidly, non-invasively, with limited or no sample preparation, at high spatial resolution (< 250 nm) and with high fidelity where the number of image pixels is limited by the number of pixels on the CCD detector. Most importantly, every image pixel has associated with it a Raman spectrum whose quality is identical to that obtained with conventional non-imaging spectrometers. These key benefits make Raman imaging practical for the analysis of real-world samples, which are chemically heterogeneous on the spatial scales of interest.

Improvements in Phase II will result in higher efficiency, and extend its usefulness into the NIR range for use with red and infra-red lasers.

Markets (anticipated after 5 years)

Based on preliminary feedback from industrial and academic researchers, the following markets are predicted for imaging Raman LCTF systems two years after the Phase II effort, when the market is mature:

Markets (Segmented by Industry)	Size/year ($M)
Semiconductor	4
Biomedical Pathology	3
Endoscopy (in vivo)	2
Polymers	3
Pharmaceutical	3
Federal labs and academics	2
Power generation	2
Environmental	1
Coatings	1

Each system will cost between $125K - $200K, and the aggregate market of $21M corresponds to approximately 100 - 125 LCTF filters/yr.

Competition

The LCTF represents the only technology that is capable of rapidly collecting high spatial/spectral resolution Raman images at high image fidelity. C.R.I. has a controlling position in the LCTF technology due to the expertise of the P.I., strong patent positions, qualified design and fabrication personnel, and extensive fabrication and testing facilities. C.R.I. has consistently demonstrated the ability to commercialize technologies developed under SBIR program support. C.R.I.'s LCTF capability in combination with Prof. Treado, a recognized leader in Raman imaging, represents a potent alliance. The concept to adapt CRI's LCTF technology specifically for Raman imaging was originally put forth by Prof. Treado, and it has been due to the close collaboration between CRI and Prof. Treado and with the support of the Phase I effort, that the technology has been developed, demonstrated and been received with such success (see the attached feature cover article describing the technology).

Principal competition will be from non-imaging methods, which is the present approach. As the advantages of the imaging methods become known to the user community, non-imaging methods will give way to C.R.I.'s technology except where imaging is of little benefit. Identifying which applications will gain from imaging methods, and which will not, is a key component of the Phase II work.

Production Plan

C.R.I. will fabricate LCTF Raman filters for sale to integrators of Raman systems such as ChemIcon, as well as to individual researchers. It does not anticipate selling complete systems, which would lie outside the areas of C.R.I.'s core strengths.

The existing C.R.I. liquid crystal production line, on which the Phase I
prototype was built, has a capacity in excess of 100 narrowband filters/yr, as
well as several thousand broadband RGB filters. A senior process scientist
was hired recently to oversee further production improvements and expansion
which will more than double this figure within the coming year. So, while
there will be a need to bring vendors on-line for the $LiNbO_3$, and to develop
internal procedures for Q/C, handling, and characterization of the narrowband
elements involved, the production of these filters will occur as part of the
overall growth of the VariSPEC filter product line.

Marketing Plan

Marketing will be through two channels: first, we will work with O.E.M. firms
and integrators such as ChemIcon, Kaiser, Renishaw, and Nicolet as they adopt
this technology in their commercial Raman systems. C.R.I. has high visibility
in this field, mainly due to Prof. Treado's work and the Phase I efforts, and
we have been contacted by most of these firms already. Most of them have a
purely technical interest at present, and will not become actively interested
from a business standpoint until applications develop which justify their
efforts to adopt and incorporate this technology. In contrast, ChemIcon has
taken a very active role in marketing this technology, and is likely to garner
the sales to 'visionaries' who see a compelling reason to use the LCTF Raman
technique.

At the same time, C.R.I. will market the LCTF filters directly to scientific
researchers, engineers, and other end-users. This is the primary market
channel for the VariSPEC filters in fluorescence, remote sensing, and other
applications, and we will use a similar blend of advertising, direct mail,
technical articles, and trade shows to promote this product as it emerges.
C.R.I. has a network of foreign representatives and agents which sell and
support our other, similarly complex, opto-electronic products worldwide, and
they will also sell the Raman LCTF.

Underpinning the market success, however, is the identification of technical
research or applications which exploit the compelling technical advantages of
LCTF Raman systems. This will drive the interest of O.E.M.s and integrators,
and will lead to development of fruitful research areas, and thus, to end-user
sales. For this, the key ingredient is an assessment of which applications
are likely to be aided by this product, which is addressed in Phase II both
through our own study of applications and through the beta-site program.

ChemIcon Inc.
The Chemical Imaging Company.

October 24, 1996

Peter V. Foukal, Ph.D.
President
Cambridge Research & Instrumentation, Inc.
21 Erie Street
Cambridge, MA 02139

Dear Peter:

In support of CRI's application for NSF Phase II SBIR funding, ChemIcon is providing the attached Follow-On Funding Commitment letter. In return for ChemIcon's important financial commitment to support Raman LCTF commercialization, we require some measure of exclusive access to the technology that we will subsidize. Thus, we are pleased to offer the following terms for your consideration:
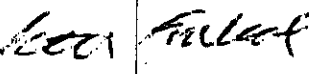
1. In return for ChemIcon's Follow-On Funding Commitment (a copy is attached) to support the Phase II SBIR entitled, "High-Definition Raman Imaging Microscope," CRI will grant ChemIcon an exclusive, Raman application specific, time-limited license to CRI's liquid crystal tunable filter (LCTF) technology that results from the NSF Phase II research. It is anticipated, at this time, that innovations that will result from the Phase II research will include, but will not be limited to, the development of red-wavelength optimized Raman LCTFs, and high throughput (Split Evans) design Raman LCTFs. The innovations will be identified by CRI, or by ChemIcon, in writing. After a suitable evaluation period, that will be agreed upon by mutual consent, it is understood that the innovative technology will only be made available to ChemIcon for resale for the time period identified below.

2. The term of the time-limited exclusive license will be 12 months. The exclusive license period will begin at a time that is determined by mutual consent. At a minimum, the beginning of the 12 month time period will commence on the date CRI has successfully delivered a resaleable Raman LCTF device that has met technical specifications identified at the time ChemIcon places a purchase order with CRI.

3. ChemIcon would be free to incorporate the Phase II LCTF technology into ChemIcon systems or to resell them directly as a licensed distributor during the Agreement term.

4. In the event of a change of ownership at CRI or ChemIcon, the terms enumerated above, as well as the Funding Commitment itself, would be assignable to any future owner or subsidiary of either organization.

If these terms are acceptable to you, please both copies of this letter, return one copy to me and retain one for your records. If you have any questions please contact me.

Submitted by: Accepted by:

Patrick J. Treado, Ph.D.   Peter V. Foukal, Ph.D.   Date
President   President

Enclosure

⊡ChemIcon Inc.
The Chemical Imaging Company.

SBIR Phase III Follow-on Funding Commitment

Whereas ChemIcon Inc. of Pittsburgh, PA desires to obtain access to the LCTF Raman imaging technology being developed by Cambridge Research & Instrumentation, Inc. (CRI) of Cambridge MA, with application development support from Patrick J. Treado and the University of Pittsburgh, ChemIcon agrees to provide long-term funding to gain access to this technology, in the amount of $200,000 against which Cambridge Research & Instrumentation will provide LCTF Raman imaging tunable filters.

This financial support is contingent upon technical progress being achieved during the Phase II effort, substantially in compliance with the Technical Objectives and Milestones contained herein as Section E and Appendix 1. In addition, this financial support is contingent on CRI's LCTF Raman imaging tunable filter technology being cost competitive with alternative Raman imaging technology, that may exist at some time during the term of this funding commitment. In addition, this financial support is contingent on the market acceptance of ChemIcon Raman microscopes that employ LCTF Raman imaging tunable filter technology.

Release of the funds will be over a period not to exceed 24 months beginning at the conclusion of the Phase II period of performance, and may begin sooner by mutual agreement, as technical progress warrants.

We certify to the best of our knowledge that this funding commitment will be used by the NSF in evaluating the commercial potential of CRI's innovation and therefore, will be a significant factor in determining whether the SBIR Phase II proposal will be funded. We further understand that willfully making a false statement or concealing a material fact in this commitment or any other communication submitted to the NSF is a criminal offense.

This commitment and support are offered and accepted by:

PATRICK J. TREADO
_____
(name)

PRESIDENT
_____
(title)

_____
(signature)

10/24/96
_____
(date)

Peter Foukal
_____
(name)

President
_____
(title)

_____
(signature)

10/25/96
_____
(date)

University of Pittsburgh

Faculty of Arts and Sciences
Department of Chemistry

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October 28, 1996

Mr. Peter Miller
Cambridge Research & Instrumentation, Inc.
21 Erie Street
Cambridge, MA 02139

Dear Mr. Miller:

I have long-standing research interests in Raman chemical imaging and its application to the analysis of materials. CRI's liquid crystal tunable filter (LCTF) technology represents a revolutionary advancement in imaging spectrometer technology that is ideally suited to Raman imaging microscopy.

I am providing this letter as a consultant in support of CRI's Phase II SBIR application to the National Science Foundation entitled "High-Definition Raman Imaging Microscope." My role in this project is to assist in the development of liquid crystal tunable filters (LCTFs) and their application to Raman imaging. Through my involvement with industrial and clinical end users of Raman chemical imaging I will also contribute to the commercialization of the technology. My consultant rate is $420/day and I will provide 60 days of effort over the term of the anticipated grant.

I look forward to CRI's continued success in the development of high quality LCTF technology for Raman imaging.

Sincerely,

Patrick J. Treado
Assistant Professor

US006734962B2

(12) **United States Patent**
Treado et al.

(10) **Patent No.:** US 6,734,962 B2
(45) **Date of Patent:** May 11, 2004

(54) **NEAR INFRARED CHEMICAL IMAGING MICROSCOPE**

(75) Inventors: **Patrick J. Treado**, Pittsburgh, PA (US); **Matthew Nelson**, Pittsburgh, PA (US); **Scott Keitzer**, Export, PA (US)

(73) Assignee: **ChemImage Corporation**, Pittsburgh, PA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 201 days.

(21) Appl. No.: **09/976,391**

(22) Filed: **Oct. 12, 2001**

(65) **Prior Publication Data**

US 2002/0113210 A1 Aug. 22, 2002

Related U.S. Application Data

(60) Provisional application No. 60/239,969, filed on Oct. 13, 2000.

(51) Int. Cl.[7] G01J 3/44
(52) U.S. Cl. 356/301; 356/51; 356/326; 356/331; 250/339.05
(58) Field of Search 356/301, 51, 310, 356/326, 328, 330–334, 73; 250/339.05, 339.02, 339.01, 339.07, 339, 458.1, 459.1, 461.1, 462.2; 382/284

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Primary Examiner—Frank G. Font
Assistant Examiner—Layla Lauchman
(74) *Attorney, Agent, or Firm*—Buchanan Ingersoll, P.C.

(57) **ABSTRACT**

A chemical imaging system is provided which uses a near infrared radiation microscope. The system includes an illumination source which illuminates an area of a sample using light in the near infrared radiation wavelength and light in the visible wavelength. A multitude of spatially resolved spectra of transmitted, reflected, emitted or scattered near infrared wavelength radiation light from the illuminated area of the sample is collected and a collimated beam is produced therefrom. A near infrared imaging spectrometer is provided for selecting a near infrared radiation image of the collimated beam. The filtered images are collected by a detector for further processing. The visible wavelength light from the illuminated area of the sample is simultaneously detected providing for the simultaneous visible and near infrared chemical imaging analysis of the sample. Two efficient means for performing three dimensional near infrared chemical imaging microscopy are provided.

16 Claims, 6 Drawing Sheets
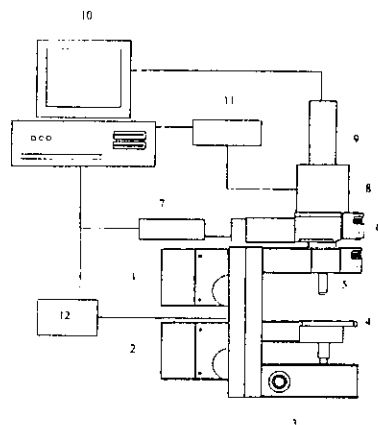
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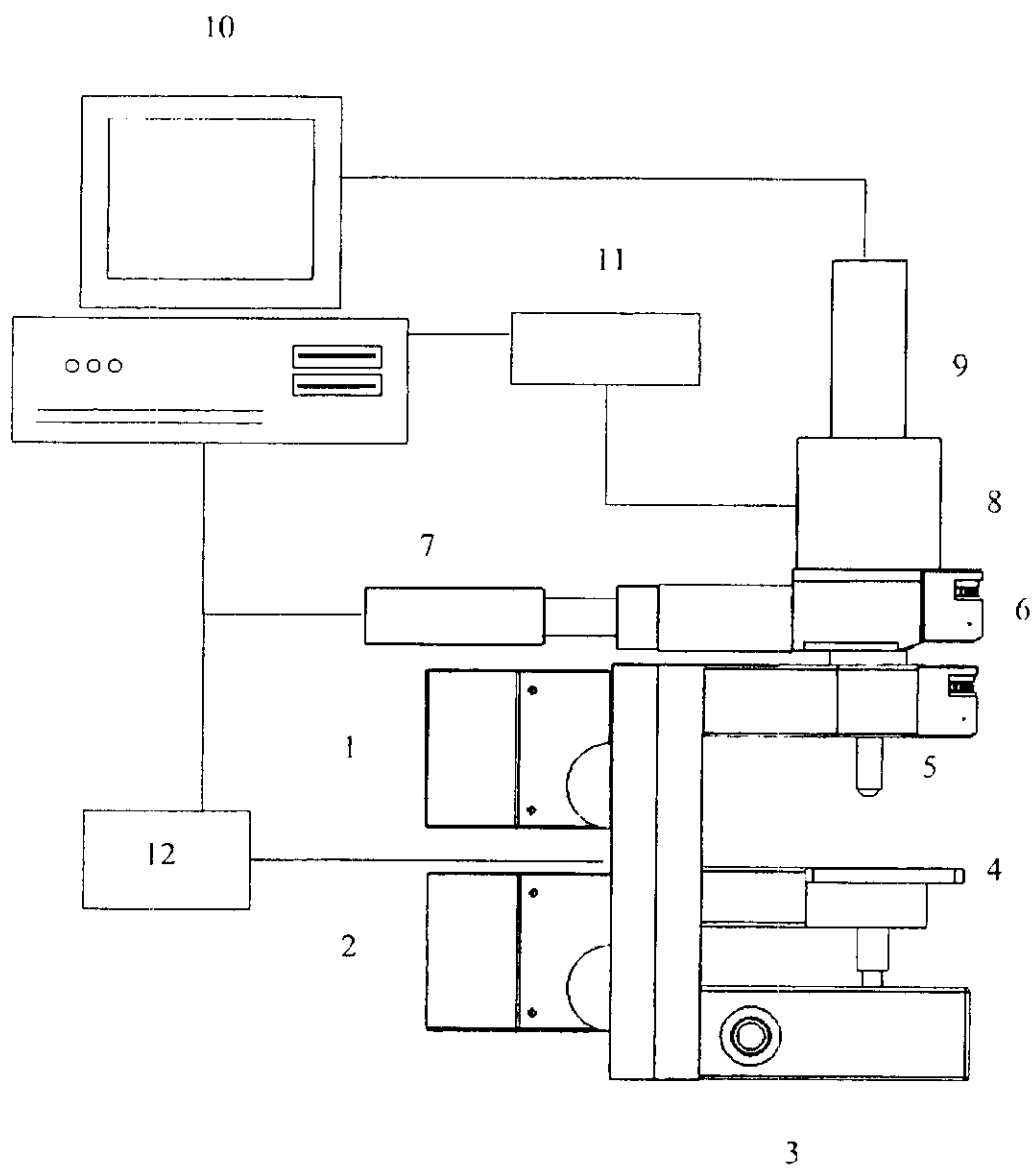
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Figure 1

Visualization   Spatial
                                 Analysis
Quantitative
Analysis

Qualitative
Analysis   Chemical
                                            Image
                The Chemical   Measurement
                Image Analysis
Preprocessing   Cycle   Automated
                                         Image
                                         Analysis

Data Acquisition SAMPLE
                     SOLUTION   Results
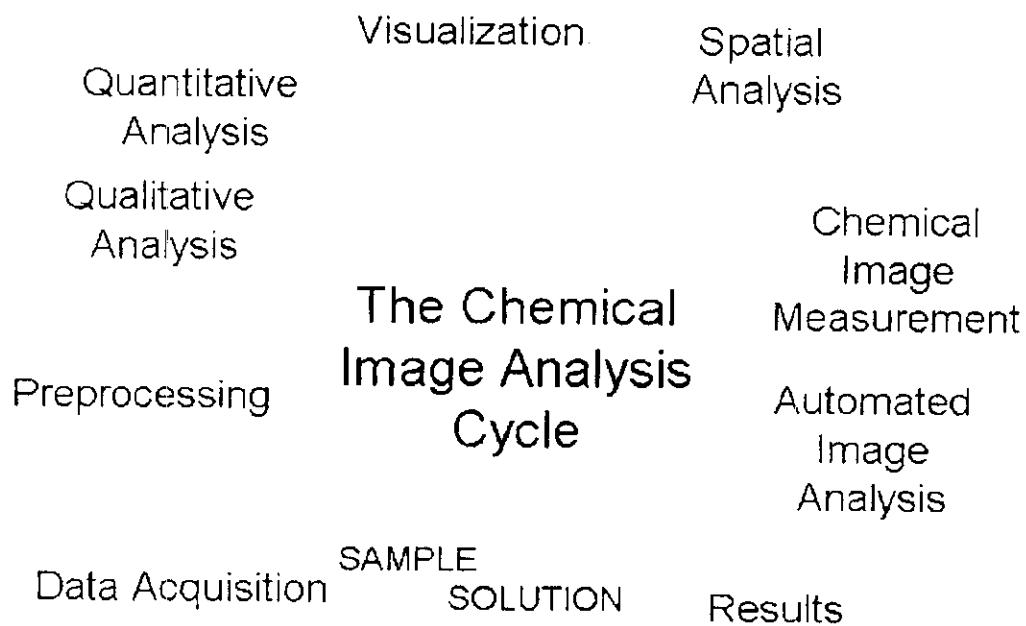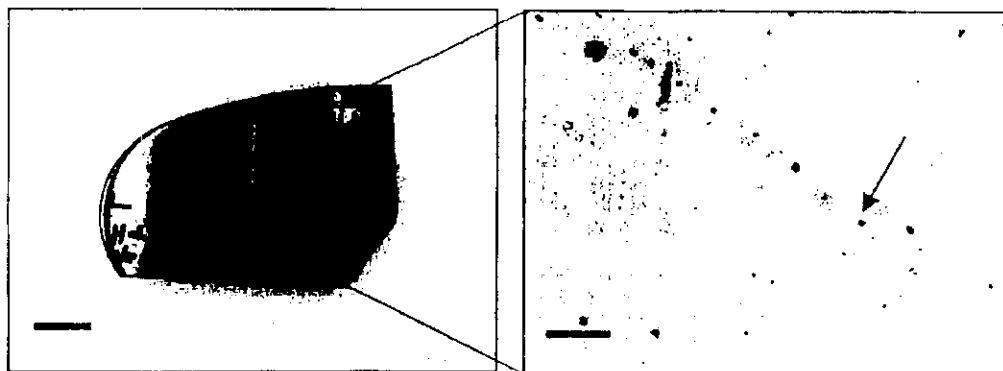
Figure 2

Figure 3
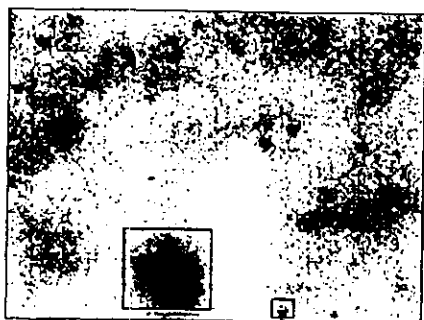
Figure 4

Figure 5A

Figure 5B

Figure 5C
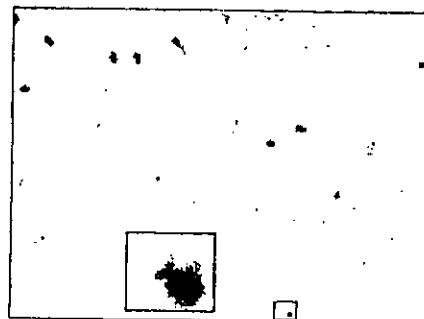
Figure 5D

Figure 6
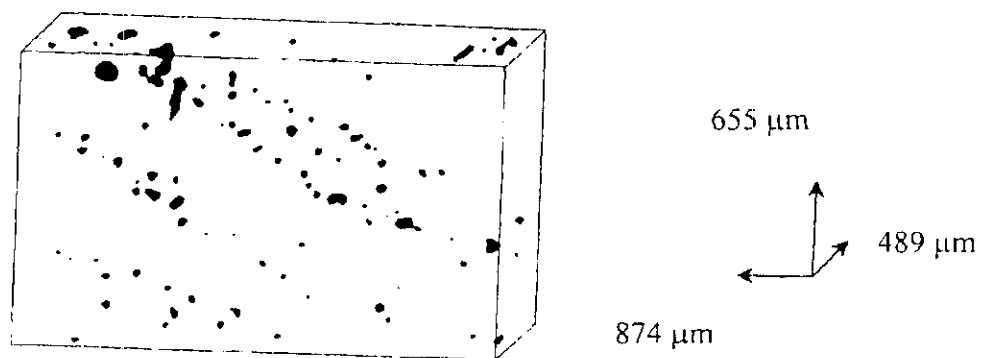
655 μm

489 μm

874 μm
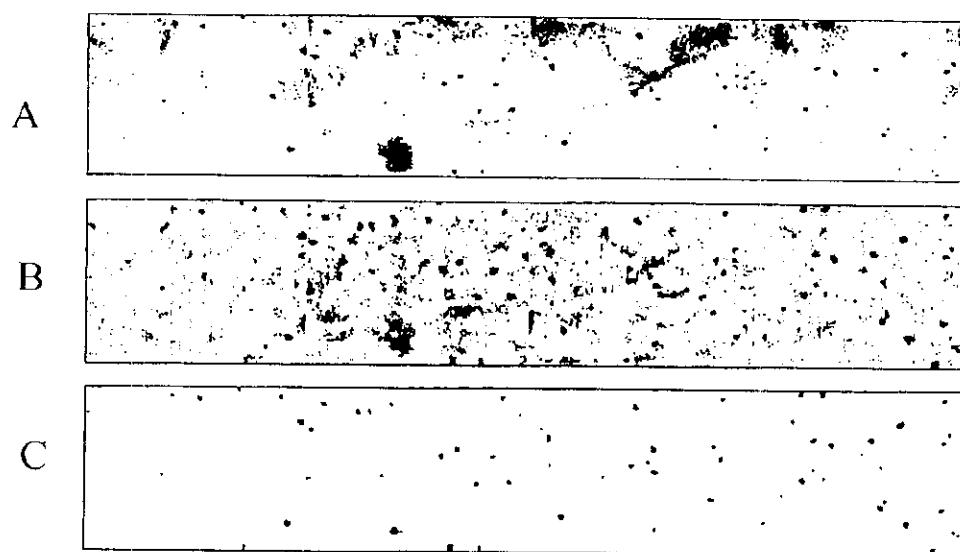
Figure 7

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NEAR INFRARED CHEMICAL IMAGING MICROSCOPE

This application claims the benefit of U.S. Provisional Application No. 60/239,969, entitled "Near Infrared Chemical Imaging Microscope" filed Oct. 13, 2000.

This work is supported by the National Institute of Standards and Technology (NIST) under the Advanced Technology Program (ATP) award (Contract Number 70NANB8H4021)

FIELD OF INVENTION

The present invention is related to near-infrared (NIR) microscopes for spectroscopic and image analysis, and, in particular, to microscopes useful for both NIR spectroscopy, NIR chemical imaging and NIR volumetric chemical imaging.

BACKGROUND OF THE INVENTION

NIR spectroscopy is a mature, non-contact, non-destructive analytical characterization tool that has been widely applied to a broad range of materials. The NIR region of the electromagnetic spectrum encompasses radiation with wavelengths of 0.78 to 2.5 μm (12,800 to 4,000 cm^{-1}). NIR spectra result from the overtone and combination bands of fundamental mid-infrared (MIR) bands. Among the many desirable characteristics, NIR is used to rapidly obtain both qualitative and quantitative information about the molecular makeup of a material. Digital imaging, on the other hand, provides a means to obtain optical (i.e., spatial—morphological, topographical, etc.) information about a material. By combining the spatial information obtained from digital imagery and the spectral information obtained from NIR spectroscopy, the chemical makeup of complex material matrices can be mapped out in both two and three spatial dimensions. NIR chemical imaging combines NIR spectroscopy and digital imaging for the molecular-specific analysis of materials. A NIR chemical imaging microscope apparatus employing NIR absorption molecular spectroscopy for materials characterization is disclosed.

State-of-the-Art Instrumentation

NIR microscopes are used to obtain NIR absorption, transmittance or reflectance spectra (e.g., NIR microspectra) from samples ranging in size between 1 and 1000 μm. These instruments are typically equipped with a digital camera to visually locate a region of interest on a sample upon which a NIR light beam from a Fourier transform (FT) spectrometer is focused. Reflective optics are used to direct the transmitted or reflected light from the sample to a NIR detector. The output is a NIR absorption spectrum collected in transmittance or reflectance mode.

NIR chemical imaging can be considered an extension of NIR microspectroscopy. Much of the imaging performed since the development of the first NIR microprobes has involved spatial scanning of samples beneath NIR microscopes in order to construct NIR "maps" of surfaces. In point by point scanning with NIR microscopes, the NIR light beam is focused onto the surface of a sample or apertured to illuminate a small region of a sample and a spectrum from each spatial position is collected. Images are obtained by rastering the sample through the focused or apertured NIR light beam and the spectra recorded are then reconstructed to form an image. Although point scanning produces images based on NIR contrast, long experimental times are common since the duration of the experiment is proportional to the number of image pixels. As a direct result, point scan images

are captured at low image definition, which relates directly to the limited utility of the technique as an imaging tool for the routine assessment of material morphology. The spatial resolution of the image is limited by the size of the NIR illumination spot on the sample (no less than 1 μm) and the rastering mechanism, which requires the use of moving mechanical parts that are challenging to operate reproducibly.

NIR imaging cameras have been used in photography for decades. Until recently, however, it has not been easily accessible to those not versed in traditional photographic processes. By using optical filters (e.g., cold filters) that block the visible wavelengths (0.4–0.78 μm), charge-coupled devices (CCDs) used in digital cameras and camcorders can be used to sense NIR light out to around 1100 nm. Other regions of the NIR spectrum can be viewed using devices such as indium gallium arsenide (InGaAs—0.9 μm to 1.7 μm) and indium antimonide (InSb—1.0 μm to 5.0 μm) focal plane array (FPA) detectors. These integrated wavelength NIR imaging approaches allow one to study relative light intensities of objects over broad ranges of the NIR spectrum, but useful chemical information is unattainable without the use of some type of discrete wavelength filtering device.

The use of dielectric interference filters in combination with NIR FPAs is one method in which chemical information can be obtained from a sample. To form NIR chemical images, a NIR light beam is defocused to illuminate a wide field of view and the reflected or transmitted light from the illuminated area is imaged onto a two-dimensional NIR detector. A selection of discrete dielectric interference filters provided in a filter wheel, or a linearly variable or circularly variable format can be positioned in front of a broadband NIR light source, or in front of the NIR FPA itself in order to collect NIR wavelength resolved images. Typically, the use of several fixed bandpass filters is required to access the entire NIR spectrum. The spatial resolution of the NIR image approaches that of the optical microscope, while spectral resolution of several nanometers has been demonstrated. Key limitations of the dielectric filter approach include the need for a multitude of discrete filters to provide appreciable free spectral range, or the reliance on moving mechanical parts in employing continuously tunable dielectric interference filters as a requirement to form wavelength resolved images. While moving mechanical assemblies can be engineered they add cost and complexity to NIR chemical imaging systems. Alternatives to moving mechanical assemblies are generally more cost effective and provide performance advantages.

Acousto-optic tunable filters (AOTFs) have been employed as no-moving-parts imaging spectrometers for NIR imaging. The AOTF is a solid-state device that is capable of functioning from the UV to the mid-IR depending on the choice of the filter's crystal material. Operation of the AOTF is based on the interaction of light with a traveling acoustic sound wave in an anisotropic crystal medium. The incident light is diffracted with a narrow spectral bandpass when an rf signal is applied to the device. By changing the applied rf frequency under computer control the spectral passband can be tuned rapidly with the benefit of non-moving parts.

For use in NIR chemical imaging, AOTFs have distinct limitations. AOTFs have imaging performance that is degraded appreciably from diffraction-limited conditions due to dispersion effects and image shifting effects. Furthermore, AOTFs suffer from temperature instability and exhibit nonlinear properties that complicate their use as imaging spectrometers.

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An aim of NIR chemical imaging technology development has been to develop a NIR imaging technique that combines diffraction-limited spatial resolution with high spectral resolution. NIR chemical imaging techniques have only recently achieved a degree of technological maturity that allow the collection of high resolution (spectral and spatial) data with the advent of the liquid crystal (LC) imaging spectrometers. In general, LC devices provide diffraction-limited spatial resolution. The spectral resolution of the LC imaging spectrometer is comparable to that provided by dispersive monochromator and Fourier transform interferometers. In addition, LC technology provides high out of band rejection, broad free spectral range, moderate transmittance, high overall etendue and highly reproducible random access computer controlled tuning.

Under normal NIR imaging operation, LC imaging spectrometers allow NIR chemical images of samples to be recorded at discrete wavelengths (energies). A spectrum is generated corresponding to thousands of spatial locations at the sample surface by tuning the LC imaging spectrometer over a range of wavelengths and collecting NIR images systematically. Contrast is generated in the images based on the relative amounts of NIR absorption, transmittance or reflectance that is generated by the different species located throughout the sample. Since a high quality NIR spectrum is generated for each pixel location, a wide variety of chemometric analysis tools, both univariate and multivariate, can be applied to the NIR image data to extract pertinent information. Correlative multivariate routines are particularly powerful when applied to chemical images collected from samples intentionally seeded with a known standard material. This approach of incorporating calibration standards within an image field of view can be extended to quantitative chemical image analysis. In addition, digital image analysis procedures can also be applied to high image quality NIR chemical images to perform routine particle analysis in both two (2D) and three (3D) spatial dimensions. Volumetric 3D NIR chemical image analysis can be performed very effectively using numerical deconvolution computational strategies.

SUMMARY OF THE INVENTION

To address the need for a device that can provide video imaging, NIR spectroscopy and high resolution (spatial and spectral) NIR chemical imaging in two and three spatial dimensions, a novel NIR chemical imaging microscope has been developed that is NIR chemical imaging capable.

The microscope design uses NIR optimized liquid crystal (LC) imaging spectrometer technology for wavelength selection. The NIR optimized refractive microscope is used in conjunction with infinity-corrected objectives to form the NIR image on the detector with or without the use of a tube lens. An integrated parfocal analog color CCD detector provides real-time sample positioning and focusing. The color image and the NIR image are fused in software. In one configuration, the NIR microscope may be used as a volumetric imaging instrument through the means of moving the sample through focus, collecting images at varying focal depths and reconstructing a volumetric image of the sample in software, or through the means of keeping the sample fixed and changing the wavelength dependent depth of penetration in conjunction with a refractive tube lens with a well characterized chromatic effect. The output of the microscope can be coupled to a NIR spectrometer either via direct optical coupling or via a fiber optic. A Chemical Imaging Addition Method seeds the sample with a material of known composition, structure and/or concentration and then gen-

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erates the NIR image suitable for qualitative and quantitative analysis. The microscope generates NIR chemical image data that is analyzed and visualized using chemical image analysis software in a systematic and comprehensive manner. While this invention has been demonstrated on a microscope optic platform, the novel concepts are also applicable to other image gathering platforms, namely fiberscopes, macrolens systems and telescopes.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a schematic diagram of the near-infrared (NIR) chemical imaging microscope

FIG. 2 shows a diagram of the chemical imaging data analysis cycle performed in software.

FIG. 3 is a digital brightfield image of a CdZnTe semiconductor material decorated with tellurium inclusions.

FIG. 4 an NIR microscopic transmittance image of a CdZnTe semiconductor material decorated with tellurium inclusions.

FIG. 5A illustrates a raw NIR image frame of a CdZnTe wafer sample.

FIG. 5B illustrates an NIR image frame of the sample of FIG. 5A in which the threshold value for the image was set too low.

FIG. 5C illustrates an NIR image frame of the sample of FIG. 5A in which the threshold value for the image was set too high.

FIG. 5D illustrates an NIR image frame of the sample of FIG. 5A in which the threshold value for the image was set to an intermediate level.

FIG. 6A is the original raw image of four adjacent regions of interest on a CdZnTe wafer.

FIG. 6B is the background-corrected image corresponding to the four adjacent regions of interest of the CdZnTe wafer of FIG. 6A.

FIG. 6C is the binarized image corresponding to the four adjacent regions of interest of the CdZnTe wafer of FIG. 6A.

FIG. 7 is a three-dimensional view of tellurium inclusions in a CdZnTe wafer.

DETAILED DESCRIPTION OF THE INVENTION

The NIR chemical imaging microscope combines in a single platform a NIR optimized refractive optical microscope base, which is equipped with NIR optimized infinity-corrected microscope objectives, an automated XYZ translational microscope stage and quartz tungsten halogen (QTH) lamps to secure and illuminate samples for NIR spectroscopy and imaging, an analog color charge-coupled device (CCD) detector for ordinary optical image collection and digital image collection, a NIR LC imaging spectrometer for NIR chemical image wavelength selection and a room temperature or optionally cooled NIR FPA for NIR image capture.

FIG. 1 is a schematic diagram of the NIR chemical imaging microscope. NIR illumination is directed to the sample in a reflected light configuration using a QTH source or other broadband white light source, including metal halide or Xe arc lamps 1 or a transmitted light configuration using QTH or suitable NIR source 2 of an NIR optimized refractive optical microscope platform 3. The reflected or transmitted NIR light is collected from the sample positioned on the automated XYZ translational microscope stage 4 through an infinity-corrected NIR optimized microscope objective 5.

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Ordinary optical imagery of the sample can be obtained using a mirror or beamsplitter or prism arrangement inserted into turret 6 and collecting an image with an analog or digital color or monochrome charge-coupled device (CCD) or CMOS detector 7. In NIR chemical imaging mode, the magnified NIR image is coupled through a NIR LC imaging spectrometer 8 and collected on a room temperature or cooled NIR focal plane array (FPA) detector 9. The FPA is typically comprised of indium gallium arsenide (InGaAs), but may be comprised of other NIR sensitive materials, including platinum silicide (PtSi), indium antimonide (InSb) or mercury cadmium telluride (HgCdTe). Using a beam-splitting element inserted into turret 6, NIR and ordinary optical imagery can be collected with an analog mono-chrome or color CCD detector 7 and NIR FPA 9 simulta-neously.

A central processing unit 10, typically a Pentium computer, is used for NIR chemical image collection and processing. The analog color CCD 7, NIR FPA 9, automated XYZ translational microscope stage 4 controlled via a controller 12 and NIR LC imaging spectrometer 8 (through LC imaging spectrometer controller 11) are operated with commercial software, such as Acquisition Manager (Chemlcon Inc.) in conjunction with ChemImage (Chemlcon Inc.).

By introducing a polarization sensitive beam splitting element in the optical path prior to the NIR LC imaging spectrometer 8 (not shown in schematic diagram), a portion of the NIR light from the sample may be coupled to a remote NIR spectrometer (also not shown in schematic diagram).

Preferably, NIR optimized liquid crystal (LC) imaging spectrometer technology is used for wavelength selection. The LC imaging spectrometer may be of the following types: Lyot liquid crystal tunable filter (LCTF); Evans Split-Element LCTF; Solc LCTF; Ferroelectric LCTF; Liq-uid crystal Fabry Perot (LCFP); or a hybrid filter technology comprised of a combination of the above-mentioned LC filter types or the above mentioned filter types in combina-tion with fixed bandpass and bandreject filters comprised of dielectric, rugate, holographic, color absorption, acousto-optic or polarization types.

One novel component of this invention, is that a NIR optimized refractive microscope is used in conjunction with infinity-corrected objectives to form the NIR image on the detector without the use of a tube lens. The microscope can be optimized for NIR operation through inherent design of objective and associated anti-reflective coatings, condenser and light source. To simultaneously provide high numerical apertures the objective should be refractive. To minimize chromatic aberration, maximize throughput and reduce cost the conventional tube lens can be eliminated, while having the NIR objective form the NIR image directly onto the NIR focal plane array (FPA) detector, typically of the InGaAs type. The FPA can also be comprised of Si, SiGe, PtSi, InSb, HgCdTe, PdSi, Ge, analog vidicon types. The FPA output is digitized using an analog or digital frame grabber approach.

An integrated parfocal analog CCD detector provides real-time sample positioning and focusing. An analog video camera sensitive to visible radiation, typically a color or monochrome CCD detector, but may be comprised of a CMOS type, is positioned parfocal with the NIR FPA detector to facilitate sample positioning and focusing with-out requiring direct viewing of the sample through conven-tional eyepieces. The video camera output is typically digi-tized using a frame grabber approach.

The color image and the NIR image are fused using software. While the NIR and visible cameras often generate

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images having differing contrast, the sample fields of view can be matched through a combination of optical and software manipulations. As a result, the NIR and visible images can be compared and even fused through the use of overlay techniques and correlation techniques to provide the user a near-real time view of both detector outputs on the same computer display. The comparitive and integrated views of the sample can significantly enhance the under-standing of sample morphology and architecture. By com-paring the visible, NIR and NIR chemical images, additional useful information can be acquired about the chemical composition, structure and concentration of species in samples.

The NIR microscope can be used as a volumetric imaging instrument through the means of moving the sample through focus in the Z, axial dimension, collecting images in and out of focus and reconstructing a volumetric image of the sample in software. For samples having some volume (bulk materials, surfaces, interfaces, interphases), volumetric chemical imaging in the NIR has been shown to be useful for failure analysis, product development and routine quality monitoring. The potential also exists for performing quan-titative analysis simultaneous with volumetric analysis. Volumetric imaging can be performed in a non-contact mode without modifying the sample through the use of numerical confocal techniques, which require that the sample be imaged at discrete focal planes. The resulting images are processed and reconstructed and visualized. Computional optical sectioning reconstruction techniques based on a variety of strategies have been demonstrated, including nearest neighbors and iterative deconvolution.

An alternative to sample positioning combined with com-putation reconstruction is to employ a tube lens in the image formation path of the microscope which introduces chro-matic aberration. As a result the sample can be interrogated as a function of sample depth by exercising the LC imaging spectrometer, collecting images at different wavelengths which penetrate to differing degrees into bulk materials. These wavelength dependent, depth dependent images can be reconstructed to form volumetric images of materials without requiring the sample to be moved, again through application of computational optical sectioning reconstruc-tion algorithms.

The output of the microscope can be coupled to a NIR spectrometer either via direct optical coupling or via a fiber optic cable. This allows conventional spectroscopic tools to be used to gather NIR spectra for traditional, high speed spectral analysis. The spectrometers can be of the following types: fixed filter spectrometers; grating based spectrom-eters; Fourier Transform spectrometers; or Acousto-Optic spectrometers.

A novel method that is readily employed by the disclosed microscope invention is a method described as the Chemical Imaging Addition Method which involves seeding the sample with a material of known composition, structure and/or concentration and then generating the NIR image suitable for qualitative and quantitative analysis. The Chemical Imaging Addition Method is a novel extension of a standard analytical chemical analysis technique, the Stan-dard Addition Method. A common practice in quantitative chemical analysis is to construct a standard calibration curve which is a plot of analytical response for a particular technique as a function of known analyte concentration. By measuring the analytical response from an unknown sample, an estimate of the analyte concentration can then be extrapo-lated from the calibration curve. In the Standard Addition Method, known quantities of the analyte are added to the

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samples and the increase in analytical response is measured. When the analytical response is linearly related to concentration, the concentration of the unknown analyte can be found by plotting the analytical response from a series of standards and extrapolating the unknown concentration from the curve. In this graph, however, the x-axis is the concentration of added analyte after being mixed with the sample. The x-intercept of the curve is the concentration of the unknown following dilution. The primary advantage of the standard addition method is that the matrix remains constant for all samples.

While the Standard Addition Method is used specifically for quantitative analysis, the Chemical Imaging Addition Method can be used for qualitative and quantitative analysis. The Chemical Imaging Addition Method relies upon spatially isolating analyte standards in order to calibrate the Chemical Imaging analysis. In chemical imaging, thousands of linearly independent, spatially-resolved spectra are collected in parallel of analytes found within complex host matrices. These spectra can then be processed to generate unique contrast intrinsic to analyte species without the use of stains, dyes, or contrast agents. Various spectroscopic methods including near-infrared (NIR) absorption spectroscopy can be used to probe molecular composition and structure without being destructive to the sample. Similarly, in NIR chemical imaging the contrast that is generated reveals the spatial distribution of properties revealed in the underlying NIR spectra.

The Chemical Imaging Addition Method can involve several data processing steps, typically including, but not limited to:

1. Ratiometric correction in which the sample NIR image is divided by the background NIR image to produce a result having a floating point data type.

2. The divided image is normalized by dividing each intensity value at every pixel in the image by the vector norm for its corresponding pixel spectrum. Where the vector norm is the square root of the sum of the squares of pixel intensity values for each pixel spectrum. Normalization is applied for qualitative analysis of NIR chemical images. For quantitative analysis, normalization is not employed, but relies instead on the use of partial least squares regression (PLSR) techniques.

3. Correlation analysis, including Euclidian Distance and Cosine correlation analysis (CCA) are established multivariate image analysis techniques that assess similarity in spectral image data while simultaneously suppressing background effects. More specifically, CCA assesses chemical heterogeneity without the need for training sets, identifies differences in spectral shape and efficiently provides chemical image based contrast that is independent of absolute intensity. The CCA algorithm treats each pixel spectrum as a projected vector in n-dimensional space, where n is the number of wavelengths sampled in the image. An orthonormal basis set of vectors is chosen as the set of reference vectors and the cosine of the angles between each pixel spectrum vector and the reference vectors are calculated. The intensity values displayed in the resulting CCA images are these cosine values, where a cosine value of 1 indicates the pixel spectrum and reference spectrum are identical, and a cosine value of 0 indicates the pixel spectrum and the reference spectrum are orthogonal (no correlation). The dimensions of the resulting CCA image is the same as the original image because the orthonormal basis set provides n reference vectors, resulting in n CCA images.

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4. Principal component analysis (PCA) is a data space dimensionality reduction technique. A least squares fit is drawn through the maximum variance in the n-dimensional dataset. The vector resulting from this least squares fit is termed the first principal component (PC) or the first loading. After subtracting the variance explained from the first PC, the operation is repeated and the second principal component is calculated. This process is repeated until some percentage of the total variance in the data space is explained (normally 95% or greater). PC Score images can then be visualized to reveal orthogonal information including sample information, as well as instrument response, including noise. Reconstruction of spectral dimension data can then be performed guided by cluster analysis, including without PCs that describe material or instrument parameters that one desires to amplify or suppress, depending on the needs of the sensing application.

Effective materials characterization with the disclosed NIR chemical imaging microscope invention typically requires application of a multitude of software procedures to the NIR chemical image. A schematic of the chemical image analysis cycle is shown in FIG. 2. A fairly comprehensive description of the variety of steps used to process chemical images is described below.

Until recently, seamless integration of spectral analysis, chemometric analysis and digital image analysis has not been commercially available. Individual communities have independently developed advanced software applicable to their specific requirements. For example, digital imaging software packages that treat single-frame gray-scale images and spectral processing programs that apply chemometric techniques have both reached a relatively mature state. One limitation to the development of chemical imaging, however, has been the lack of integrated software that combines enough of the features of each of these individual disciplines to have practical utility.

Historically, practitioners of chemical imaging were forced to develop their own software routines to perform each of the key steps of the data analysis. Typically, routines were prototyped using packages that supported scripting capability, such as Matlab, IDL, Grams or LabView. These packages, while flexible, are limited by steep learning curves, computational inefficiencies, and the need for individual practitioners to develop their own graphical user interface (GUI). Today, commercially available software does exist that provides efficient data processing and the ease of use of a simple GUI.

Software that meets these goals must address the entirety of the chemical imaging process. The chemical imaging analysis cycle illustrates the steps needed to successfully extract information from chemical images and to tap the full potential provided by chemical imaging systems. The cycle begins with the selection of sample measurement strategies and continues through to the presentation of a measurement solution. The first step is the collection of images. The related software must accommodate the full complement of chemical image acquisition configurations, including support of various spectroscopic techniques, the associated spectrometers and imaging detectors, and the sampling flexibility required by differing sample sizes and collection times. Ideally, even relatively disparate instrument designs can have one intuitive GUI to facilitate ease of use and ease of adoption.

The second step in the analysis cycle is data preprocessing. In general, preprocessing steps attempt to minimize contributions from chemical imaging instrument response

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that are not related to variations in the chemical composition of the imaged sample. Some of the functionalities needed include: correction for detector response, including variations in detector quantum efficiency, bad detector pixels and cosmic events; variation in source illumination intensity across the sample; and gross differentiation between spectral lineshapes based on baseline fitting and subtraction. Examples of tools available for preprocessing include ratiometric correction of detector pixel response; spectral operations such as Fourier filters and other spectral filters, normalization, mean centering, baseline correction, and smoothing; spatial operations such as cosmic filtering, low-pass filters, high-pass filters, and a number of other spatial filters.

Once instrument response has been suppressed, qualitative processing can be employed. Qualitative chemical image analysis attempts to address a simple question, "What is present and how is it distributed?". Many chemometric tools fall under this category. A partial list includes: correlation techniques such as cosine correlation and Euclidean distance correlation; classification techniques such as principal components analysis, cluster analysis, discriminant analysis, and multi-way analysis; and spectral deconvolution techniques such as SIMPLISMA, linear spectral unmixing and multivariate curve resolution.

Quantitative analysis deals with the development of concentration map images. Just as in quantitative spectral analysis, a number of multivariate chemometric techniques can be used to build the calibration models. In applying quantitative chemical imaging, all of the challenges experienced in non-imaging spectral analysis are present in quantitative chemical imaging, such as the selection of the calibration set and the verification of the model. However, in chemical imaging additional challenges exist, such as variations in sample thickness and the variability of multiple detector elements, to name a few. Depending on the quality of the models developed, the results can range from semi-quantitative concentration maps to rigorous quantitative measurements.

Results obtained from preprocessing, qualitative analysis and quantitative analysis must be visualized. Software tools must provide scaling, automapping, pseudo-color image representation, surface maps, volumetric representation, and multiple modes of presentation such as single image frame views, montage views, and animation of multidimensional chemical images, as well as a variety of digital image analysis algorithms for look up table (LUT) manipulation and contrast enhancement.

Once digital chemical images have been generated, traditional digital image analysis can be applied. For example, Spatial Analysis and Chemical Image Measurement involve binarization of the high bit depth (typically 32 bits/pixel) chemical image using threshold and segmentation strategies. Once binary images have been generated, analysis tools can examine a number of image domain features such as size, location, alignment, shape factors, domain count, domain density, and classification of domains based on any of the selected features. Results of these calculations can be used to develop key quantitative image parameters that can be used to characterize materials.

The final category of tools, Automated Image Processing, involves the automation of key steps or of the entire chemical image analysis process. For example, the detection of well defined features in an image can be completely automated and the results of these automated analyses can be tabulated based on any number of criteria (particle size, shape, chemical composition, etc). Automated chemical

imaging platforms have been developed that can run for hours in an unsupervised fashion.

This invention incorporates a comprehensive analysis approach that allows user's to carefully plan experiments and optimize instrument parameters and should allow the maximum amount of information to be extracted from chemical images so that the user can make intelligent decisions.

EXAMPLE

Overview

As the demand for high quality, low cost X-ray, γ-ray and imaging detector devices increases, there is a need to improve the quality and production yield of semiconductor materials used in these devices. One effective strategy for improving semiconductor device yield is through the use of better device characterization tools that can rapidly and nondestructively identify defects at early stages in the fabrication process. Early screening helps to elucidate the underlying causes of defects and to reduce downstream costs associated with processing defect laden materials that are ultimately scrapped. The present invention can be used to characterize tellurium inclusion defects in cadmium zinc telluride (CdZnTe) semiconductor materials based on near infrared imaging. With this approach, large area wafers can be inspected rapidly and non-destructively in two and three spatial dimensions by collecting NIR image frames at multiple regions of interest throughout the wafer using an automated NIR imaging system. The NIR image frames are subjected to image processing algorithms including background correction and image binarization. Particle analysis is performed on the binarized images to reveal tellurium inclusion statistics, sufficient to pass or fail wafers. In addition, data visualization software is used to view the tellurium inclusions in two and three spatial dimensions.

Background

The present invention has been used to automatically inspect tellurium inclusions in CdZnTe. Compound semiconductors are challenging to fabricate. There are several steps along the manufacturing process in which defects can arise. The chemical nature associated with semiconductor defects often plays a vital role in device performance. Device fabrication and device processing defects can be difficult and time consuming to measure during manufacturing. Unfortunately, defective devices are often left undiagnosed until latter stages in the manufacturing process because of the inadequacy of the metrology tools being used. This results in low production yields and high costs which can be an impediment to growth in the semiconductor device market potential.

There is a general need in the semiconductor industry for metrology technologies that can nondestructively assess semiconductor material defects and ultimately increase manufacturing yields. A potential solution is to develop a high throughput screening system capable of fusing multiple chemical imaging modalities into a single instrument. Chemical imaging combines digital imaging and molecular spectroscopy for the chemical analysis of materials. A modality of based on near-infrared (NIR) chemical imaging can be used to inspect tellurium inclusions in CdZnTe compound semiconductor materials.

CdZnTe is a leading material for use in room temperature X-ray detectors, γ-ray radiation detectors and imaging devices. Applications for these devices include nuclear diagnostics, digital radiography, high-resolution astrophysical X-ray and γ-ray imaging, industrial web gauging and nuclear nonproliferation. These devices are often decorated with microscopic and macroscopic defects limiting the yield

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of large-size, high-quality materials. Defects commonly found in these materials include cracks, grain boundaries, twin boundaries, pipes, precipitates and inclusions. CdZnTe wafers are often graded based on the size and number of Te inclusion defects present.

The definition used by Rudolph and Muhlberg for tellurium inclusions (i.e., tellurium-rich domains in the 1–50 μm size range that originate as a result of morphological instabilities at the growth interface as tellurium-rich melt droplets are captured from the boundary layer ahead of the interface) has been adopted and is used herein. There have been numerous studies on the composition and distribution of tellurium inclusions in CdZnTe material. It has been demonstrated that the presence of tellurium inclusions can impair the electronic properties of CdZnTe materials— consequently degrading the end-product device performance.

The current procedure used by low volume semiconductor manufacturers for characterizing tellurium inclusions in CdZnTe is labor intensive, susceptible to human error and provides little information on inclusions in the 1–5 μm size scale. Inclusions are viewed and counted manually by a human operator using an IR microscope platform. When an inclusion is identified that is suspected to exceed a specified size limit, a Polaroid film photograph is taken. An overlay of a stage micrometer is laid over the photograph to determine the size. This analysis is relatively time consuming, often taking several minutes to characterize a region of interest from a large wafer.

The present invention can be used for automated characterization of microscale tellurium inclusions in CdZnTe based on volumetric NIR chemical imaging. The system takes advantage of the fact that CdZnTe is transparent to infrared wavelengths (>850 nm). When viewing CdZnTe with an infrared focal plane array (IR-FPA) through a NIR LC imaging spectrometer, tellurium inclusions appear as dark, absorbing domains. The invention images wafers in two and three spatial dimensions capturing raw infrared images at each region of interest. Images are automatically background equilibrated, binarized and processed. The processed data provides particle statistical information such as inclusion counts, sizes, density, area and shape. The system provides a rapid method for characterizing tellurium inclusions as small as 0.5 μm while virtually eliminating the subjectivity associated with manual inspection.

Sample Description

Tellurium-rich CdZnTe samples were produced by a commercial supplier (eV Products) for analysis. Samples containing high tellurium inclusion densities were purposely acquired to effectively demonstrate the capabilities of the automated tellurium inclusions mapping system. The CdZnTe materials were grown by the Horizontal Bridgeman (HB) method and contained a nominal zinc cation loading concentration of 4% and an average etch pit density of 4×10^4/cm². The materials displayed a face A <111> orientation and were polished on both sides. Sample thicknesses ranged from approximately 1 mm to 15 mm. No further sample preparation was necessary for the automated tellurium inclusion mapping analysis.

Data Collection

Volumetric maps of the tellurium inclusions in the CdZnTe samples were obtained by first placing the sample on the XYZ-translational stage of the automated mapping system. NIR image frames were then captured through the LC imaging spectrometer at a wavelength that maximized the Te precipitate contrast relative to the surrounding CdZnTe matrix in the X-Y direction at multiple regions of

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interest across the samples. Depth profiling was achieved by translating the sample focus under the microscope at user-defined increments. This process was then repeated in an iterative fashion until the entire wafer was characterized.

Data Processing

Once imaging data was collected, ChemImage was used to process the data. For each wafer, the software generates a background-corrected grayscale image, a binarized image using the threshold value selected for each frame of the image, a montage view of the binarized image and particle statistics. The particle statistics table includes information such as particle counts, particle sizes, particles densities, and a number of geometrical parameters such as particle area and particle aspect ratios.

NIR Imaging

FIGS. 3 and 4, respectively, show a digital macro brightfield image and a raw NIR microscopic transmittance image of a CdZnTe semiconductor material with numerous tellurium inclusions. The left half of the wafer has been polished. The tellurium inclusions appear as dark spots in the microscopic NIR image. The raw NIR microscopic image was acquired using the automated near-infrared tellurium inclusion volumetric mapping system.

Background Correction and Image Binarization

The automated particle analysis begins by applying a background correction preprocessing routine to the raw image frames. One of the biggest problems with the raw images collected is the gradually varying background across each image frame. As a result, a particle in one area of a frame may have a higher intensity value than the background of another area of that frame.

FIGS. 5A–5D illustrate the difficulty associated with selecting a threshold value for an image with a widely varying background. In FIGS. 5A–5D, regions 1 and 2 have mean intensity values of approximately 2600 and 1950, respectively. The whole of region 1 is primarily a particle whereas region 2 is primarily background with a small particle in the center. FIG. 5A shows a raw NIR image frame collected from a single region of interest in a CdZnTe wafer. At wavelengths longer than approximately 850 nm, CdZnTe is transparent while tellurium inclusions remain opaque. A NIR image of the sample is light where there are no precipitates and dark where there are precipitates. In FIG. 5B, the threshold value is set low enough (value=1520) that the particle in region 2 is correctly identified, but most of the remaining particles are not found. In FIG. 5C, the threshold value is set high enough (value=2470) so that all particles are detected. Unfortunately, a large area of the frame is incorrectly identified as one very large particle. FIG. 5D displays the case in which the threshold is set to an intermediate value (value=1960). Many of the particles are correctly identified, but the particle in region 2 is identified as being larger than it actually is.

To address this issue, a background correction step is used to force the background to be essentially constant across a given image frame. The procedure applies a moving window across the image frame and smoothes the resulting background before subtracting it from the frame. Other operations such as low pass filtering and selective removal of bad camera pixels are also applied.

The second step in the automated particle analysis is the selection of the threshold value resulting in the binarized image which best reflects the number and size of particles actually present in the sample being imaged. A human operator would typically approach this problem by trying multiple threshold values and comparing the resulting binarized images to the actual image to see which binarized

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image best matches their perception of the particles in the actual image. The algorithm employed by the NIR chemical imaging microscope system takes essentially the same approach. A series of threshold values are used to generate binarized images. Each binarized image is submitted to a routine that finds the particles present in the image. A set of particle morphology rules was developed to determine the point at which the threshold value identifies the particles consistent with results obtained by a trained human operator. This threshold value is then further refined with using derivative operations.

FIGS. 6A–6C show montage views of raw, background-corrected, and binarized NIR image frames, respectively, corresponding to four adjacent regions of interest from a CdZnTe wafer. A visual inspection of these images suggests that the particle analysis adequately identifies the particles in an automated fashion.

Volumetric Reconstruction and Visualization

It is of particular interest to the semiconductor manufacturing industry to view defects, including tellurium inclusions in this example, in a three dimensional volumetric view. Individual binarized image frames generated at discrete axial planes of focus have been reconstructed into a volumetric view allowing users to view tellurium inclusions in three-dimensional space.

FIG. 7 shows a 3D volumetric view of tellurium inclusions in CdZnTe generated from 50 individual image slices. FIG. 7 is constructed using a nearest neighbors computational approach for volume reconstruction. Improved results can be obtained using more sophisticated strategies that deconvolve the entire image volume using iterative deconvolution approaches. The staring time of the sensor used to gather the volumetric data was less than 1 sec. The total acquisition time for the data generated in this figure was well under a minute. Note how the inclusions tend to form in planes described as veils. These veils are believed to be subgrain boundaries within the CdZnTe material. Grain boundaries provide low energy nucleation sites for the inclusions to form during the growth process.

Table 1 provides tabulated statistical information on the volumetric data shown in FIG. 7.

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titative information about tellurium inclusions present in CdZnTe wafers in two and three spatial dimensions. This system boasts improved spatial resolution (~ 0.5 μm) compared to systems currently used by many semiconductor manufacturers and it virtually eliminates the subjectivity associated with human counting and sizing measurements. Whole wafers are capable of being characterized in minutes.

While in the above example, the present invention has been demonstrated in connection with the characterization of semiconductors, it is to be expressly understood that the present invention can also be used in the characterization of other materials including, but not limited to, food and agricultural products, paper products, pharmaceutical materials, polymers, thin films and in medical uses.

Although present preferred embodiments of the invention have been shown and described, it should be distinctly understood that the invention is not limited thereto but may be variously embodied within the scope of the following claims.

We claim:

1. A near infrared radiation chemical imaging system comprising:
   a) an illumination source for illuminating an area of a sample using light in the near infrared radiation wavelength;
   b) a device for collecting a spectrum of near infrared wavelength radiation light transmitted, reflected, emitted or scattered from said illuminated area of said sample and producing a collimated beam therefrom;
   c) a near infrared imaging spectrometer for selecting a near infrared radiation image of said collimated beam; and
   d) a detector for collecting said filtered near infrared images.

2. The system of claim 1 wherein said illumination source is one of a quartz tungsten halogen lamp, a tunable laser, a metal halide lamp, and a xenon arc lamp.

3. The system of claim 1 wherein said device for collecting is one of a refractive type infinity-corrected near infrared optimized microscope objective, a refractive fixed tube length microscope objective, and a reflecting microscope objective.

TABLE I

			Particle Statistics Slice Number and Depth (μm)			
Parameters	0 (0)	10 (89.77)	20 (189.52)	30 (289.26)	40 (389.01)	50 (488.75)
# of Inclusions	25	30	27	24	25	36
Mean Diameter (μm)	12.12	11.38	12.75	15.70	12.89	13.73
Density (Inclusions/cm^2)	4368	5241	4717	4193	4368	6289
Area (μm^2)	97.48	73.78	91.67	119.25	96.29	98.15
Perimeter (μm)	40.40	37.32	43.27	50.72	41.93	43.98
Shape Factor	0.60	0.60	0.58	0.53	0.60	0.55
Maximum Chord Length	12.12	11.38	12.75	15.70	12.89	13.73
Feret 1 Diameter	9.17	9.56	11.33	12.64	10.48	10.16
Feret 2 Diameter	10.26	9.01	10.10	12.18	10.37	11.60
Aspect Ratio	1.02	1.19	1.16	1.08	1.02	0.95

Defects such as tellurium inclusions affect the electrical properties in CdZnTe semiconductor materials, degrading end-product device performance. Having the ability to rapidly and non-invasively identify and quantify tellurium inclusion defects at critical stages in the fabrication process provides semiconductor manufacturers with information that will enable them to optimize the manufacturing process and reduce production costs. The Automated NIR Volumetric Mapping System described here is capable of providing such information. The system provides qualitative and quan-

4. The system of claim 1 wherein said near infrared imaging spectrometer is selected from the group consisting of Lyot liquid crystal tunable filters; Evans Split-Element liquid crystal tunable filters; Sole liquid crystal tunable filters; Ferroelectric liquid crystal tunable filters; Liquid crystal Fabry Perot filters; a hybrid filter formed from a combination of liquid crystal tunable filters; and a combination of a liquid crystal tunable filter and a fixed bandpass and bandreject filters.

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5. The system of claim 1 wherein said detector is a near infrared radiation focal plane array detector.

6. The system of claim 5 wherein said detector is selected from the group consisting of indium gallium arsenide, platinum silicide, indium antimonide, palladium silicide, indium germanide, and mercury cadmium telluride.

7. The system of claim 1 further comprising a visible wavelength imagery system.

8. The system of claim 7 wherein said visible imagery system comprises:

   a) an illumination source for illuminating an area of said sample using light in the visible optical wavelengths; and

   b) a device for detecting said visible wavelength light from said illuminated area of said sample.

9. The system of claim 8 wherein said device for detecting said visible wavelength light comprises an analog and digital detector based on at least one of a silicon charge-coupled device detector and a silicon CMOS detectors.

10. The system of claim 8 further comprising a processor for producing a near infrared radiation chemical image of said sample.

11. The system of claim 8 further comprising an algorithm for combining the near infrared and visible image data.

12. A chemical imaging system comprising:

   a) an illumination source for illuminating an area of a sample using light in the near infrared radiation wavelength and light in the visible wavelength;

   b) a device for collecting a spectrum of near infrared wavelength radiation light transmitted, reflected, emitted or scattered from said illuminated area of said sample and producing a collimated beam therefrom;

   c) a near infrared imaging spectrometer for selecting a near infrared radiation image of said collimated beam;

   d) detector for collecting said filtered near infrared images; and

   e) a device for detecting said visible wavelength light from said illuminated area of said sample.

13. A chemical imaging method comprising the steps of:

   a) illuminating an area of a sample using light in the near infrared radiation wavelength and light in the visible wavelength;

   b) collecting a spectrum of near infrared wavelength radiation light transmitted, reflected, emitted or scattered from said illuminated area of said sample and producing a collimated beam therefrom;

   c) filtering said collimated beam to produce a near infrared radiation image of said collimated beam while

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simultaneously detecting said optical wavelength light from said illuminated area of said sample;

   d) collecting said filtered near infrared images; and

   e) processing said collected near infrared images to produce a chemical image of said sample.

14. A method for producing a volumetric image of a sample comprising the steps of:

   a) incorporating a refractive image formation optic exhibiting a chromatic response in the optical path of the microscope before the near infrared detector;

   b) collecting images of said sample at a plurality of near infrared wavelengths through said objective at a fixed focus condition; and

   c) processing said collected images to reconstruct a depth resolved image of said sample.

15. A method for chemically analyzing a sample comprising the steps of:

   a) seeding said sample with a plurality of analytes having at least one of a known composition, structure and concentration;

   b) collecting a plurality of spatially-resolved spectra for said plurality of analytes;

   c) producing a plurality of chemical images of said sample containing said plurality of anayltes; and

   d) processing said plurality of chemical images to generate a chemical image of said sample.

16. The method of claim 15 wherein said processing step comprises at least one of:

   a) correcting the image by dividing a near infrared image of said sample by a near infrared image of a background of said image to produce a resulting ratioed image;

   b) normalizing the divided image by dividing each intensity value at every pixel in the image by the vector norm for its corresponding pixel spectrum, said vector norm being the square root of the sum of the squares of pixel intensity values for each pixel spectrum;

   c) processing said image using a cosine correlation analysis method wherein each pixel spectrum is treated as a projected vector in n-dimensional space, wherein n is the number of wavelengths sampled in the image; and

   d) processing said image using a principal component analysis method wherein a least squares fit is drawn through the maximum variance in the n-dimensional dataset.

* * * * *

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December 29, 2004

VIA FACSIMILE (412) 241-7311
CONFIRMATION WITH ENCLOSURES VIA FEDERAL EXPRESS

Dr. Wes Hutchison
ChemImage Corporation
7301 Penn Avenue
Pittsburg, PA 15208

Re: U.S. Patent No. 6,734,962
       Our ref.: 34250-29

Dear Dr. Hutchison:

We are intellectual property counsel for Cambridge Research & Instrumentation, Inc. ("CRI"). We write concerning ChemImage Corporation's ("ChemImage") U.S. Patent No. 6,734,962 ("'962 patent"). In this regard, we have reviewed the exchange of emails between Ted Les of CRI and you concerning this patent.

You may recall that previously ChemImage executed a covenant not to sue in favor of CRI with respect to ChemImage's (then ChemIcon) U.S. Patent No. 6,002,476. We believe that the present situation is similar to that one, and that here too it would be in everyone's best interest for ChemImage to execute a covenant not to sue in favor of CRI with respect to the '962 patent. As more fully explained below, the '962 patent is infirm at least because Peter Miller should have been named as a co-inventor (in which event CRI would have had a joint ownership interest in the '962 patent).

Presently, CRI's ability to market its products is being impaired by the existence of ChemImage's '962 patent, and for that reason CRI cannot accept the status quo. However, ChemImage's execution of a covenant not to sue with respect to the '962 patent in favor of CRI would resolve all of CRI's concerns. On the other hand, if ChemImage does not agree to execute a covenant not to sue, CRI will have no choice but to take appropriate action to remove the '962 patent as an obstacle.

Peter Miller is a co-inventor of subject matter claimed in the '962 patent

Systems fully anticipating one or more of the claims of the '962 patent were described in an SBIR Phase II proposal entitled "High-Definition Raman Imaging Microscope", which was submitted by CRI to the National Science Foundation in 1996, i.e., about four years before the earliest application for the '962 patent was filed. Peter Miller was the principal investigator ("P.I.") on this proposal and Patrick Treado, a named inventor on the '962 patent, was a consultant. As the P.I., Mr. Miller was primarily responsible for the technical direction of the research. Funding for this Phase II proposal was awarded to CRI, and the work was concluded successfully. If the work

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resulting from that grant is patentable, then Mr. Miller should have been named as an inventor on the '962 patent, in which event CRI would have had an ownership interest in the patent.

A copy of the SBIR proposal as submitted to the National Science Foundation by CRI on or about October 30, 1996 is attached as Exhibit 1. Dr. Treado provided a letter indicating his willingness to serve as a consultant on the project, which is included in the proposal and appears in Exhibit 1. Dr. Treado's letter is dated October 28, 1996, at which time Dr. Treado was an employee of the University of Pittsburgh. Dr. Treado left the University after submission of the proposal and during the course of the work on the project.

Chemlcon and CRI had an agreement concerning the commercialization rights to systems developed under the SBIR proposal which is signed by Dr. Treado and by the President of CRI, Peter Foukal. That agreement is dated October, 1996 and is also included in Exhibit 1. At no time did CRI assign to Chemlcon or to Dr. Treado CRI's patent rights in the work resulting from the project.

On page 6 the SBIR proposal lists six research objectives, two of which are:

> - to develop a near-IR (NIR) version of the Raman LCTF which extends the long-wavelength operating limit from the present value of 700 nm to a minimum of 1050 nm;

and

> - to construct and characterize a minimum of one visible-range and one NIR-range Raman LCTF which incorporate these improvements

These objectives were part of a comprehensive plan to achieve tangible results, which included -

i)   utilizing improved designs to improve the transmission of Raman LCTF filters
ii)   developing a near-IR version of the Raman LCTF for the range 700 – 1050 nm
iii) constructing and characterizing at least one visible range and one NIR Raman LCTF
iv)   assessing the benefits of LCTF in various applications
v)   making at least one of the LCTF systems available to collaborators
vi)   developing data analysis methods to exploit the newly-available information

As shown by the title, objectives, and plan of work, the SBIR proposal of Exhibit 1 addressed the problem of chemical imaging; it did so utilizing LCTFs, which are imaging spectrometer filters; and the chemical imaging was done in the near-infrared range. Thus it concerns the precise subject matter addressed in the '962 patent..

This is more fully demonstrated by comparing the text of claim 1 of the '962 patent to the work accomplished pursuant to the SBIR proposal. Claim 1 of the '962 patent reads:

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1. A near infrared radiation chemical imaging system comprising:

a) an illumination source for illuminating an area of a sample using light in the near infrared radiation wavelength;

b) a device for collecting a spectrum of near infrared wavelength radiation light transmitted, reflected, emitted or scattered from said illuminated area of said sample and producing a collimated beam therefrom;

c) a near infrared imaging spectrometer for selecting a near infrared radiation image of said collimated beam; and

d) a detector for collecting said filtered near infrared images.

Taking each claim element in turn:

a) an illumination source for illuminating an area of a sample using light in the near infrared radiation wavelength;

The SBIR proposal uses a laser light source to illuminate a sample. See Figure 2 on page 27 of Exhibit 1 and the text on page 4.

Page 9 of the SBIR proposal of Exhibit 1 provides the following work task and explanation:

"ii) to develop a near-IR (NIR) version of the Raman LCTF which extends the long-wavelength operating limit from the present value of 700 nm to a minimum of 1050 nm;

The P.I. has developed a high-efficiency NIR polarizer during a previous NASA project directed towards remote sensing projects. It is a special version of a commercially-available polarizer, optimized for the NIR range extending to the end of silicon CCD response at 1050 nm. While conventional (sic) sheet-polarizer is heavily-dyed and has relatively low transmission as a result, the transmission of this material is greater than 90% at all NIR wavelengths, and above 94% over the range 800-1050 nm. It is thus ideal for use with laser diode sources at 780 nm and longer."

The '962 patent defines the NIR range as 780 – 2500 nm (see column 1, line 25), which includes the NIR wavelengths contemplated in the SBIR proposal.

Thus, this element of claim 1 is fully met by the SBIR proposal.

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b) a device for collecting a spectrum of near infrared wavelength radiation light transmitted, reflected, emitted or scattered from said illuminated area of said sample and producing a collimated beam therefrom;

The optical system used to collect light from the sample in the SBIR proposal is described on page 4: "The system was constructed around an Olympus BHSM-2 microscope, as shown in Figure 2". Page 4 also explains that this microscope uses an infinity-corrected objective which is used to collect light scattered by the sample: "Raman scatter is collected by the same infinity-corrected objective ..."

Thus, the microscope of Figure 2 uses an infinity-corrected objective to collect light scattered from the area of the sample illuminated by the light source. An infinity-corrected objective produces a collimated beam, as claim 3 of the '962 patent makes clear, wherein element b of claim 1 is defined as a member of the group consisting of "a refractive type infinity-corrected near infrared optimized microscope objective . . .". See also column 3, lines 50-52 of the '962 patent ("The NIR optimized refractive microscope is used in conjunction with infinity-corrected objectives to form the NIR image on the detector with or without the use of a tube lens.").

Indeed, such infinity-corrected objectives had been used in previous work published by Dr. Treado and CRI as authors, including "Imaging Spectrometers for Fluorescence and Raman Microscopy: Acousto-Optic and Liquid Crystal Tunable Filters", Morris et. al., Applied Spectroscopy **48** (7), pp 857-866. This article notes that "image collection is provided by infinity-corrected plan-achromat ... objectives". The optical diagram in that article shows that the light collected from the sample is collimated as it passes through the LCTF.

c) a near infrared imaging spectrometer for selecting a near infrared radiation image of said collimated beam

As the patent (e.g., claim column 5, lines 31-35 and claim 4) makes clear, an LCTF is an imaging spectrometer for selecting a near infrared image from a collimated beam, and indeed is an ideal choice for this purpose.

The proposal uses an LCTF for the same purpose. As set forth in the proposal at, for example, pages 9 and 10, CRI personnel conceived, designed, developed, and built an LCTF operating in the 700 – 1050 nm range, i.e., in the near infrared region as defined in the '962 patent.

d) a detector for collecting said filtered near infrared images.

The proposal also describes this element on page 4: "Raman scatter is collected by the same infinity-corrected objective . . .The Raman signal then passes through the LCTF and is imaged onto a cooled CCD camera . . .". As the proposal indicates, silicon CCD response extends to 1050 nm, i.e., in the near infrared region.

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The use of a detector to produce an image of the sample is repeated throughout the proposal, including the Project Summary page C-1 and pages 3-5.

As the foregoing demonstrates, at least claim 1 is fully met by the SBIR proposal and the work that ensued from it. That being the case, Peter Miller should have been named as a co-inventor on the patent, and the failure to name him either renders the patent invalid, or at the very least entitles CRI to an unrestricted right to practice the invention claimed in the patent.

For at least this reason, the '962 patent is unenforceable against CRI, and the most efficient way to address this issue is for ChemImage to execute a covenant not to sue in favor of CRI with respect to the '962 patent.

　*　　　　*　　　　*　　　　*　　　　*　　　　*

While CRI would prefer to resolve this issue on mutually acceptable terms, as noted the '962 patent is impacting CRI's commercial activities, and in the absence of prompt resolution CRI intends to take steps to remove the '962 patent as an obstacle. Accordingly, please let us have your response to this letter **by no later than January 6, 2004.**

We look forward to hearing from you.

Very truly yours,
COHEN, PONTANI, LIEBERMAN & PAVANE

Martin Pavane (jc)

Martin B. Pavane

MBP/jc
Enc.

cc: Cambridge Research & Instrumentation, Inc. (via mail w/enc.)

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Philadelphia, PA 19103-2921
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COUNSELORS AT LAW

Daniel H. Golub
215.963.5055
dgolub@morganlewis.com

January 20, 2005

VIA FACSIMILE (212)972-5487
Martin B. Pavane, Esquire
Cohen Pontani Lieberman & Pavane
551 Fifth Avenue
New York, NY 10176

Re: U.S. Patent No. 6,734,962

Dear Mr. Pavane:

We have your letter dated December 29, 2004, which asserts that "the '962 patent is infirm at least because Peter Miller should have been named as a co-inventor."

We have reviewed the SBIR proposal included with your letter, and strongly disagree that it constitutes evidence that Peter Miller was a co-inventor of the '962 patent. While the SBIR proposal suggests that Peter Miller and Dr. Treado collaborated on certain technology, much of the subject matter described in the SBIR proposal was invented by Dr. Treado before any such collaboration took place. The fact that Peter Miller and Dr. Treado may have collaborated on ideas previously invented by Dr. Treado does not in any way diminish Dr. Treado's position as the sole inventor of the technology that he (and ChemIcon) brought to the SBIR proposal.

As you know, a party challenging inventorship in connection with an issued patent bears the burden of proof by clear and convincing evidence. The SBIR proposal provided with your December 29, 2004 letter does not come close to meeting this exacting standard. The fact that Peter Miller is listed as an investigator on the SBIR proposal does not, as you suggest, represent evidence that he is an inventor of technology described therein. Typically, a person asserting that he should be named as patent inventor provides written documentation such as lab notebook entries, evidencing that party's conception and reduction to practice of the technology at issue.

Philadelphia Washington New York Los Angeles San Francisco Miami Pittsburgh Princeton
Chicago Palo Alto Dallas Harrisburg Irvine Boston London Paris Brussels Frankfurt Tokyo

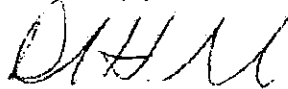**Morgan Lewis**
COUNSELORS AT LAW

Martin B. Pavane, Esquire
January 20, 2005
Page 2

To the extent that you have evidence such as laboratory notebook entries that you believe are relevant to the claims of the '962 patent, we ask that you provide us copies of these materials so that we can consider them. However, based on our understanding of the facts, and the materials that you have provided to date, we can only conclude that Peter Miller's claim of co-inventorship of the '962 patent is utterly without merit.

In view of the above, ChemImage will certainly not be advised to execute the covenant not to sue that CRI has proposed in connection with this matter.

Should you have any questions about this letter, or wish to discuss this matter further, please feel free to contact me.

Very truly yours,

Daniel H. Golub

JS 44   (Rev. 11/04)

CIVIL COVER SHEET

The JS 44 civil cover sheet and the information contained herein neither replace nor supplement the filing and service of pleadings or other papers as required by law, except as provided by local rules of court. This form, approved by the Judicial Conference of the United States in September 1974, is required for the use of the Clerk of Court for the purpose of initiating the civil docket sheet.   (SEE INSTRUCTIONS ON THE REVERSE OF THE FORM.)

I. (a) PLAINTIFFS
Peter J. Miller, Clifford Hoyt, and
Cambridge Research and Instrumentation, Inc.

DEFENDANTS CLERKS OFFICE
Patrick Treado and Chemimage, Corp.

FILED

2005 FEB 24 P 2: 35

U.S. DISTRICT COURT
DISTRICT OF MASS.

(b) County of Residence of First Listed Plaintiff   __Essex__
(EXCEPT IN U.S. PLAINTIFF CASES)

County of Residence of First Listed Defendant   Allegheny County
(IN U.S. PLAINTIFF CASES ONLY)
NOTE: IN LAND CONDEMNATION CASES, USE THE LOCATION OF THE
LAND INVOLVED.

(c) Attorney's (Firm Name, Address, and Telephone Number)
Erin McLaughlin   (617) 856-8297
Brown Rudnick Berlack Israels, One Financial Ctr.
Boston, MA

Attorneys (If Known)

II. BASIS OF JURISDICTION   (Place an "X" in One Box Only)

☐ 1 U.S. Government
     Plaintiff

☒ 3 Federal Question
     (U.S. Government Not a Party)

☐ 2 U.S. Government
     Defendant

☐ 4 Diversity
     (Indicate Citizenship of Parties in Item III)

III. CITIZENSHIP OF PRINCIPAL PARTIES (Place an "X" in One Box for Plaintiff
(For Diversity Cases Only)   and One Box for Defendant)

	PTF	DEF		PTF	DEF
Citizen of This State	☒ 1	☐ 1	Incorporated or Principal Place of Business In This State	☒ 4	☐ 4
Citizen of Another State	☐ 2	☒ 2	Incorporated and Principal Place of Business In Another State	☐ 5	☒ 5
Citizen or Subject of a Foreign Country	☐ 3	☐ 3	Foreign Nation	☐ 6	☐ 6

IV. NATURE OF SUIT   (Place an "X" in One Box Only)

CONTRACT	TORTS		FORFEITURE/PENALTY	BANKRUPTCY	OTHER STATUTES
☐ 110 Insurance	**PERSONAL INJURY**	**PERSONAL INJURY**	☐ 610 Agriculture	☐ 422 Appeal 28 USC 158	☐ 400 State Reapportionment
☐ 120 Marine	☐ 310 Airplane	☐ 362 Personal Injury -	☐ 620 Other Food & Drug	☐ 423 Withdrawal	☐ 410 Antitrust
☐ 130 Miller Act	☐ 315 Airplane Product	Med. Malpractice	☐ 625 Drug Related Seizure	28 USC 157	☐ 430 Banks and Banking
☐ 140 Negotiable Instrument	Liability	☐ 365 Personal Injury -	of Property 21 USC 881		☐ 450 Commerce
☐ 150 Recovery of Overpayment	☐ 320 Assault, Libel &	Product Liability	☐ 630 Liquor Laws	**PROPERTY RIGHTS**	☐ 460 Deportation
& Enforcement of Judgment	Slander	☐ 368 Asbestos Personal	☐ 640 R.R. & Truck	☐ 820 Copyrights	☐ 470 Racketeer Influenced and
☐ 151 Medicare Act	☐ 330 Federal Employers'	Injury Product	☐ 650 Airline Regs.	☒ 830 Patent	Corrupt Organizations
☐ 152 Recovery of Defaulted	Liability	Liability	☐ 660 Occupational	☐ 840 Trademark	☐ 480 Consumer Credit
Student Loans	☐ 340 Marine	**PERSONAL PROPERTY**	Safety/Health		☐ 490 Cable/Sat TV
(Excl. Veterans)	☐ 345 Marine Product	☐ 370 Other Fraud	☐ 690 Other		☐ 810 Selective Service
☐ 153 Recovery of Overpayment	Liability	☐ 371 Truth in Lending	**LABOR**	**SOCIAL SECURITY**	☐ 850 Securities/Commodities/
of Veteran's Benefits	☐ 350 Motor Vehicle	☐ 380 Other Personal	☐ 710 Fair Labor Standards	☐ 861 HIA (1395ff)	Exchange
☐ 160 Stockholders' Suits	☐ 355 Motor Vehicle	Property Damage	Act	☐ 862 Black Lung (923)	☐ 875 Customer Challenge
☐ 190 Other Contract	Product Liability	☐ 385 Property Damage	☐ 720 Labor/Mgmt. Relations	☐ 863 DIWC/DIWW (405(g))	12 USC 3410
☐ 195 Contract Product Liability	☐ 360 Other Personal	Product Liability	☐ 730 Labor/Mgmt.Reporting	☐ 864 SSID Title XVI	☐ 890 Other Statutory Actions
☐ 196 Franchise	Injury		& Disclosure Act	☐ 865 RSI (405(g))	☐ 891 Agricultural Acts
REAL PROPERTY	**CIVIL RIGHTS**	**PRISONER PETITIONS**	☐ 740 Railway Labor Act	**FEDERAL TAX SUITS**	☐ 892 Economic Stabilization Act
☐ 210 Land Condemnation	☐ 441 Voting	☐ 510 Motions to Vacate	☐ 790 Other Labor Litigation	☐ 870 Taxes (U.S. Plaintiff	☐ 893 Environmental Matters
☐ 220 Foreclosure	☐ 442 Employment	Sentence	☐ 791 Empl. Ret. Inc.	or Defendant)	☐ 894 Energy Allocation Act
☐ 230 Rent Lease & Ejectment	☐ 443 Housing/	**Habeas Corpus:**	Security Act	☐ 871 IRS—Third Party	☐ 895 Freedom of Information
☐ 240 Torts to Land	Accommodations	☐ 530 General		26 USC 7609	Act
☐ 245 Tort Product Liability	☐ 444 Welfare	☐ 535 Death Penalty			☐ 900Appeal of Fee Determination
☐ 290 All Other Real Property	☐ 445 Amer. w/Disabilities -	☐ 540 Mandamus & Other			Under Equal Access
	Employment	☐ 550 Civil Rights			to Justice
	☐ 446 Amer. w/Disabilities -	☐ 555 Prison Condition			☐ 950 Constitutionality of
	Other				State Statutes
	☐ 440 Other Civil Rights				

V. ORIGIN   (Place an "X" in One Box Only)

☒ 1 Original Proceeding ☐ 2 Removed from State Court ☐ 3 Remanded from Appellate Court ☐ 4 Reinstated or Reopened ☐ 5 Transferred from another district (specify) ☐ 6 Multidistrict Litigation ☐ 7 Appeal to District Judge from Magistrate Judgment

VI. CAUSE OF ACTION
Cite the U.S. Civil Statute under which you are filing (Do not cite jurisdictional statutes unless diversity):
35 U.C.S. et seq.

Brief description of cause:
patent dispute

VII. REQUESTED IN COMPLAINT:
☐ CHECK IF THIS IS A CLASS ACTION UNDER F.R.C.P. 23
DEMAND $
CHECK YES only if demanded in complaint:
JURY DEMAND: ☐ Yes   ☒ No

VIII. RELATED CASE(S) IF ANY
(See instructions):   JUDGE _____   DOCKET NUMBER _____

DATE   2/24/05
SIGNATURE OF ATTORNEY OF RECORD

FOR OFFICE USE ONLY

RECEIPT # _____   AMOUNT _____   APPLYING IFP _____   JUDGE _____   MAG. JUDGE _____

UNITED STATES DISTRICT COURT
DISTRICT OF MASSACHUSETTS

1. Title of case (name of first party on each side only)____Peter J. Miller v. Patrick Tirado____

2. Category in which the case belongs based upon the numbered nature of suit code listed on the civil cover sheet. (See local rule 40.1(a)(1)).

   ☐ I.   160, 410, 470, R.23, REGARDLESS OF NATURE OF SUIT.

   X II.   195, 368, 400, 440, 441-444, 540, 550, 555, 625, 710, 720, 730,   *Also complete AO 120 or AO 121
            740, 790, 791, 820*, 830*, 840*, 850, 890, 892-894, 895, 950.   for patent, trademark or copyright cases

   ☐ III. 110, 120, 130, 140, 151, 190, 210, 230, 240, 245, 290, 310,
            315, 320, 330, 340, 345, 350, 355, 360, 362, 365, 370, 371,
            380, 385, 450, 891.

   ☐ IV.   220, 422, 423, 430, 460, 510, 530, 610, 620, 630, 640, 650, 660,
            690, 810, 861-865, 870, 871, 875, 900.

   ☐ V.   150, 152, 153.

3. Title and number, if any, of related cases. (See local rule 40.1(g)). If more than one prior related case has been filed in this district please indicate the title and number of the first filed case in this court.

   None

4. Has a prior action between the same parties and based on the same claim ever been filed in this court?

                                                          YES ☐   NO ☒

5. Does the complaint in this case question the constitutionality of an act of congress affecting the public interest?   (See 28 USC §2403)

                                                          YES ☐   NO ☒

   If so, is the U.S.A. or an officer, agent or employee of the U.S. a party?

                                                          YES ☐   NO ☐

6. Is this case required to be heard and determined by a district court of three judges pursuant to title 28 USC §2284?

                                                          YES ☐   NO ☒

7. Do all of the parties in this action, excluding governmental agencies of the united states and the Commonwealth of Massachusetts ("governmental agencies"), residing in Massachusetts reside in the same division? - (See Local Rule 40.1(d)).

                                                          YES ☐   NO ☒

   A.   If yes, in which division do all of the non-governmental parties reside?

        Eastern Division ☐   Central Division ☐   Western Division ☐

   B.   If no, in which division do the majority of the plaintiffs or the only parties, excluding governmental agencies, residing in Massachusetts reside?

        Eastern Division ☒   Central Division ☐   Western Division ☐

8. If filing a Notice of Removal - are there any motions pending in the state court requiring the attention of this Court? (If yes, submit a separate sheet identifying the motions)

                                                          YES ☐   NO ☒

(PLEASE TYPE OR PRINT)

ATTORNEY'S NAME _____Erin McLaughlin_____

ADDRESS Brown Rudnick Berlack Israels , One Financial Center, Boston, MA 02110

TELEPHONE NO. _____617-856-8297_____

(Coversheetlocal.wpd - 10/17/02)